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الجامعة الأردنيـة
كلية الدراسات العليا

التاريـخ: ٢ / ١١ / ٢٠١١

نموذج رقم (١٨)
اقرار والتزام بقوانين الجامعة الأردنية وأنظمتها وتعليماتها لطلبة الماجستير

أنا الطالب: نسرين فوزي محمد الجياجات الرقم الجامعي: ٨٠٦١٠٥٨٥
التخصص: تغذية وتصنيع غذائي الكلية: الزراعة

اعلن بأنني قد التزمت بقوانين الجامعة الأردنية وأنظمتها وتعليماتها وقراراتها السارية المفعول المتعلقة باعداد رسائل الماجستير والدكتوراة عندما قمت شخصيا" باعداد رسالتي / اطروحتي بعنوان: ....
Effect of Virgin olive oil on Antioxidant Capacity and Glycemic and Lipidemic Control in Streptozotocin-Induced Diabetic Adult Rats.

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توقيع الطالب: .... التاريخ: ٢ / ١١ / ٢٠١١

تعتمد كلية الدراسات العليا
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التوقيع .... التاريخ ....

EFFECT OF VIRGIN OLIVE OIL ON ANTIOXIDANT CAPACITY AND GLYCEMIC AND LIPIDEMIC CONTROL IN STREPTOZOTOCIN-INDUCED DIABETIC ADULT RATS

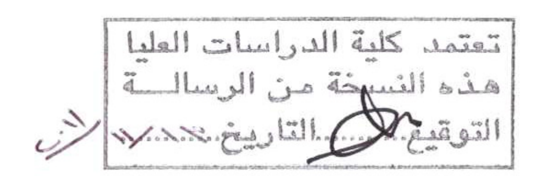
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This Thesis was Submitted in Partial Fulfillment of the Requirements for the Master's Degree of Science in Nutrition and Food Technology

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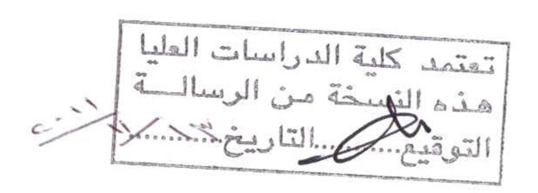
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Committee Decision

This Thesis (Effect of Virgin Olive Oil on Antioxidant Capacity and Glycemic and Lipidemic Control in Streptozotocin-Induced Diabetic Adult Rats) was Successfully Defended and Approved on 27th, 2011.

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Dedication

To my parents... who put me one the road by their endless favors
To my beloved husband, Suliman, who enlighten my life with love and hope

To my brothers and sisters
To my lovely son Bashar

To those who wished me success....

Acknowledgment

It is my pleasure to extend my special thanks and appreciation to my supervisor Dr. Musa N Ahmad for his continuous encouragement and guidance during research.

My gratitude and acknowledgment to the examination committee Members: Dr. Malike Haddadin, Dr. Faisal Mohammed, Dr. Hiba Bawadi.

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List of Abbreviations

ADA	American Diabetes Association
AFI	Accumulated Food Intake
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
AWI	Accumulated Water Intake
CDC	Centers of Disease Control and Prevention
CHO	Carbohydrate
DDH_2O	Double Deionized Water
DOS	Department of Statistics
FER	Food Efficiency Ratio
FFA	Free Fatty Acid
GSH	Glutathione
HDL-C	High Density Lipoprotein -cholesterol
HKJ	Hashemite Kingdom of Jordan
$Hx\text{-}Fe^{III}$	Metmyoglobin
IDDM	Insulin Dependent Diabetes Mellitus
KCal	Kilocalorie
LDL-C	Low Density Lipoprotein -cholesterol
LSD	Least Significant Difference
LW	Liver weight/100g boy weight
mmol	Millimole
MUFA	Monounsaturated Fatty Acids
N	Normality
NADH	Reduced Nicotinamide Adenine Dinucleotide
NADPH	Reduced Nicotinamide Adenine Dinucleotide Phosphate
NIDDM	Non Insulin Dependent Diabetes Mellitus
P.V	Peroxide Value
PUFA	Polyunsaturated Fatty Acids
ROS	Reactive Oxygen Species
rpm	Revolution per minute
SAS	Statistical Analysis System
SEM	Standard Error of Mean
SFA	Saturated Fatty Acid
SFA	Saturated fatty acid
STZ	Streptozotocin
TAC	Total Antioxidant Capacity
TAS	Total Antioxidant Status
TC	Total Cholesterol
TG	Triglycerides
TRL	Triacylglcerol-Rich Lipoproteins
VLDL	Very Low Density Lipoprotein
WC	Weight Change
WHO	World Health Organization
x	Maximum Peroxide value allowed in Jordanian Standard to virgin olive oil
$X\text{-}[Fe^{IV}=0]$	Ferrylmyoglobin

EFFECT OF VIRGIN OLIVE OIL ON ANTIOXIDANT CAPACITY AND GLYCEMIC AND LIPIDEMIC CONTROL IN STREPTOZOTOCIN-INDUCED DIABETIC ADULT RATS

By
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ABSTRACT

This study was conducted to investigate whether the consumption of diets with varying proportions of virgin and stored preparation of olive oil had an effect on the serum antioxidant capacity and glucose and lipid levels in diabetic rats. Diabetes was partially induced by intraperitoneal injection of streptozotocin (32.25 mg/kg). Virgin olive oil was exposed to accelerated storage conditions and oil preparations with 1.5x and 2.0x of the maximum recommended peroxide value (P.V) according to the Jordanian standards were obtained. Hence, virgin olive oil and stored oil preparations with P.V of 30 and 40 meqO_2/kg were used. Forty-eight male Sprague-Dawley rats (217.2 ± 4.6 g) were divided into six groups. Each group (8 rats/ group) was fed a diet containing 4% and 8% of either virgin olive oil or stored oil preparations with 1.5x P.V and 2.0x P.V for five weeks. Fasting serum levels of glucose, total cholesterol (TC), low- density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), Triglycerides (TG) and total antioxidant capacity (TAC) were determined by enzymatic- colorimetric methods using standard kit procedures. Body weights, liver weights, and accumulative food and water intakes were also recorded. Acidity, P.V and TAC of virgin olive oil and stored oil preparations were measured.

The P.V for virgin olive oil was (10±0.01meqO_2/kg), whereas for its preparations were 30 and 40 meqO_2/kg of 1.5x P.V and 2.0x P.V respectively. The acidity of virgin olive oil (0.98±0.02 oleic acid %) increased in parallel to the increase in P.V (1.24±0.01 and 1.52±0.01 oleic acid % for 1.5x P.V and 2.0x P.V preparations respectively), However, TAC of virgin olive oil (2.22±0.02 mmol/l) decreased as P.V increased (2.15±0.02 and 2.05±0.01 mmol/l for 1.5x P.V and 2.0x P.V preparations respectively).

Feeding 4% and 8% virgin olive oil and stored oil preparations with P.V of 30 and 40 meqO_2/kg to various diabetic rat groups resulted in insignificant (P>0.05) changes in body weight, accumulative food and water intakes and food efficiency ratio. Regardless of oil percent or oil P.V, similar results were obtained. Rats fed 4% stored oil preparation with P.V of 30 meqO_2/kg showed lighter (P<0.05) relative liver weight (3.5± 0.15) compared to those fed 4% virgin olive oil (4.1± 0.09) and 8% stored oil preparation with P.V of 30 meqO_2/kg (4.1±0.03). feeding 4% and 8% olive oil with various P.V did not significantly (P>0.05) affect fasting levels of serum glucose, TC, LDL-C, HDL-C, TG , ratios of LDL-C/HDL-C and LDL-C/TG and Atherogenic index. Similar results were obtained when comparing experimental groups regardless of both oil percent and P.V of oil.

Serum TAC exhibited a significant increasing ($P<0.05$) trend as oil P.V increased. feeding 8% oil with 2.0x P.V resulted in a significant ($P<0.05$) increase in serum TAC (1.18± 0.02 mmol/l) compared to feeding 4% virgin olive oil (1.05±0.03mmol/l) and 4% oil with 1.5x P.V (1.07±0.03mmol/l). Rats fed 4% virgin olive oil exhibited lower serum TAC (1.05±0.03mmol/l) compared to those fed 8% oil with 1.5x P.V (1.17±0.03 mmol/l). Serum TAC correlated positively with serum TC $r =0.39$, $P<0.01$) and TG ($r =0.35$, $P<0.05$) and negatively with food intake ($r =-0.35$, $P<0.05$) and water intake ($r =-0.42$, $P<0.01$). Serum glucose correlated positively with food intake ($r =0.33$, $P<0.05$) and water intake ($r =0.43$, $P<0.01$) and negatively with weight change ($r =-0.39$, $P<0.01$) and food efficiency ratio ($r =-0.42$, $P<0.01$). Food intake correlated well with water intake ($r= 0.81$, $P<0.01$), and weight change related markedly to food efficiency ratio ($r= 0.93$, $P<0.01$). Serum TC and LDL-C correlated negatively with weight change ($r =-0.36$, $P<0.05$; $r =-0.42$, $P<0.01$ respectively) and with food efficiency ratio ($r =-0.34$, $P<0.05$; $r =-0.42$, $P<0.01$ respectively).

It may be concluded that virgin olive oil or its stored preparations exerted little or no effect on serum levels of glucose, TC, HDL-C, LDL-C, and TG in uncontrolled streptozotocin-induced diabetes in rats. This conclusion may be also driven to effects on body weight, food intake and water intake. The respective increase in P.V and acidity followed by a parallel decrease in TAC of the experimental oil enhanced the TAC status of diabetic rats. However, the significance of the results of the present study demands further investigations.

Introduction

Diabetes mellitus is defined as a metabolic disorder of multiple aetiology, characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (WHO, 2006a). The disease causes profound alterations in the metabolic pathways regulated by insulin, a state that has several overt symptoms, such as hyperglycaemia, hyperlipideamia, ketoacidosis and body weight defects (Gerstein, 2007). Diabetes mellitus is the most common metabolic disease world wide (Wild, *et al*, .2004; Sicree and Shaw,2007). It is a major cause of death in many countries (Virally, *et al*., 2007), and presents a major health problem in Jordan (Zindah, *et al*., 2008; Ajlouni, *et al*., 2008).

Oxidative stress resulting from hyperglycaemia and hyperlipidaemia is now considered to be involved in the development of diabetes complications, particularly, cardiovascular diseases (CVD). Both conditions lead to multiple metabolic defects leading to increased production of reactive oxygen species (Chiasson, 2006). Reactive oxygen species are known to be responsible for oxidative damage of DNA, nucleotides, proteins, lipids, carbohydrates and cell membrane structure (Piwowar, *et al*., 2007). However, the cause effect relationship between oxidative stress and the development of diabetes and its complications is not yet established.

Olive oil is rich in monounsaturated fatty acids and antioxidant compounds mainly phenolic compounds, carotenoids and tocopherols (Boskou, 2000). The oil is unique as it is not produced by solvent extraction, but rather by a cold-press mechanical process that preserves its chemical nature. Olive oil has been shown to have several health benefits when taken as a part of a habitual diet (Puerta, *et al*., 2007). However, heat and air –light exposure of the oil may alter its composition especially fatty acid and

antioxidant contents, a matter that may affect olive oil health benefits (Fito, *et al*., 2005; Naz, *et al*., 2005).

Fresh olive oil has been associated with the decrease in the risks of CVD, since it decreases low-density lipoprotein cholesterol (LDL-C), increases high-density lipoprotein cholesterol (HDL-C), and reduces oxidative stress (Carluccio, *et al*., 2007; Oliveras-Lopez, *et al*., 2008). Olive oil-rich diets have been shown to be associated with the reduction in LDL oxidation, the hallmark for CVD development (Fito, *et al*., 2005). The incidence of CVD is relatively low in the Mediterranean countries due to diet being rich in olive oil (Hu, 2004). Results of more recent epidemiologic investigations support the view that higher consumption of antioxidants is linked to better control glycemic markers in individuals, and may prevent diabetes development (Panagiotakos, *et al*., 2007; Psaltopoulou, *et al*., 2009).

Olive oil has been shown to protect LDL from lipid peroxidation in *in vitro* experiments (Owen, *et al.,* 2000). Animal studies suggest a protective effect of olive oil phenols on LDL oxidation (Visioli, *et al.,* 2000). However, the information from controlled intervention trials in humans or animals, which provides first level of scientific evidence, regarding the in vivo effects of olive oil, either virgin or stored, (i.e. exposed to air, light and heat) on the antioxidant capacity in diabetes mellitus are not available.

Based on the scientific information just presented, a hypothesis was made that pathological changes occurring in diabetes mellitus affect the homeostasis of body weight, blood glucose, lipid profile and total antioxidant capacity, and the extent of this effect may be influenced by the incorporation of olive oil either virgin or stored olive oil in the diet.

The present study aimed at examining whether the consumption of diets with varying proportions of virgin and stored olive oil (exposed to air, light and heat) has an effect on the antioxidant capacity and serum glucose and lipid levels in adult rats with streptozotocin- induced diabetes mellitus.

Literature Review

1. Diabetes Mellitus
1.1. Introduction

Diabetes mellitus is one of the most common noncommunicable diseases, and its epidemic proportion has placed it at the forefront of public health challenges currently facing the world (WHO, 2006b). More than 220 million people world wide have diabetes (WHO, 2009) and this number will reach 366 million cases by 2030 (Wild, *et al*., 2004). Diabetes mellitus have been defined as a metabolic disorder of multiple etiologies, characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effect of diabetes mellitus includes long-term damage, dysfunction and failure of various organs (WHO, 2006b).

1.2. Classification and Etiology

Diabetes mellitus is classified into four categories depending on etiology: Insulin dependent diabetes mellitus (IDDM or Type I), non-insulin dependent diabetes mellitus (NIDDM or Type II), gestational diabetes, and other conditions leading to diabetes (WHO,2006b).

Type I diabetes, which results from autoimmune β-cell destruction in the pancreas and is characterized by a complete lack of insulin production. Type I diabetes accounts for 5%-10% of all cases of diabetes (Krzystek-Korpacka*, et al* 2008). Its risk factors include autoimmune, genetic, and environmental factors (ADA, 2006). It appears most often during childhood or adolescence (CDC, 2008).

Type I diabetes is characterized by hyperglycemia, hypertriglyceridemia (chylomicrons and very low-density lipoprotein (VLDL)), and episodes of severe ketoacidosis. The hyperglycemia results from inability of the insulin dependent tissues

to utilize glucose and due to accelerated hepatic gluconeogenesis from amino acids derived from muscle protein. Lipoprotein lipase is inactivated due to absence of insulin resulting in facilitaty free fatty acids release from adipose tissue and occurrence of hypertriglyceridemia. The ketoacidosis results from increased lipolysis in adipose tissue and accelerated fatty acid oxidation in liver (Harris and Crabb, 2006). The symptoms of this type of diabetes are: polyuria, polydipsia, polyphagia, weight loss, blurred vision, recurrent vaginal or urinary tract infections, and fatigue (Daneman*.,* 2006).

Type II diabetes , which develops when there is an abnormal increased resistance to the action of insulin and the body cannot produce enough insulin to overcome the resistance (Deshpande*, et al* ., 2008). It accounts for 90%-95% of all diagnosed diabetes cases (Sicree and shaw, 2007). This form of diabetes generally begins as insulin resistance increased and because the body is unable to produce enough insulin to overcome the resistance; the pancreas may reduce the production of insulin or eventually stop producing it (Deshpande *et al*., 2008). Risk factors for type II diabetes include genetic and environmental factors, such as a family history of diabetes, old age, obesity particularly intraabdominal obesity, physical inactivity, and a prior history of gestational diabetes (Asian-Pacific Type 2 diabetes policy Group, 2002).

Gestational diabetes; defined as a form of glucose intolerance that affects some women during pregnancy (WHO, 2006a). Minority women, who are obese, with a family history of diabetes, and have had gestational diabetes in a previous pregnancy, are at higher risk than other women for developing gestational diabetes. Women who have had gestational diabetes have a 20%-50% increased risk for developing type II diabetes later in life. Glycemic control and management of women with gestational diabetes are necessary to prevent birth complications in the developing infant (CDC, 2005).

Other type of diabetes caused by specific genetic defects of β-cell function or insulin action, are diseases of the pancreas, or drugs or chemicals (ADA, 2006). An intermediate class was added to describe a clinical condition. This clinical condition is called impaired fasting glycemia, when fasting blood glucose is the measured parameter ≥100 and <126 mg/dl and impaired glucose tolerance, when blood sugar level two hours after oral glucose tolerance is measured≥140 and <200 mg/dl (CDC,2005).

1.3. Prevalence

Diabetes mellitus is a global growing problem. According to recent estimates of the WHO (2009), more than 220 million people have diabetes in the world and this number will increase to 366 million by 2030 (Wild *et al*., 2004). In the eastern Mediterranean region, there has been a rapid increase in the incidence of diabetes mellitus, and it is now the fourth leading cause of death in the region (WHO, 2006b). Approximately 1.1 million people died from diabetes in 2005, 80% of diabetes deaths took place in low and middle-income countries (WHO, 2009).

In Jordan, high prevalence of type II diabetes and impaired fasting glucose was found by Ajlouni and *et al* (2008), in which the over all prevalence was 33.2% (18.3 in males and 16.9 in females) of the age group ≥25 years. The prevalence of impaired glucose tolerance was 12.4% (8.9 in males and 7.7 in females) of the age group ≥25 years. On the other hand, type I diabetes has the lowest prevalence in the region, but is rising from 2.8 to 3.6 per 100,000 populations through 1992 to 1996 (Ajlouni *et al*., 1999).

1.4. Diagnostic Criteria

The diagnosis of diabetes in an asymptomatic individual should never be performed on the basis of a single abnormal glucose value. Verification of the diagnosis with repeat testing is required, unless an individual presents with unequivocal

hyperglycaemia, along with its classic symptoms. Table 1 shows the diagnostic values for diabetes mellitus and other categories of hyperglycaemia.

Table 1: Diagnostic values for diabetes mellitus and other categories of hyperglycaemia.[1]

	Venous plasma glucose concentration	
	mmol/l	mg/dl
Diabetes mellitus		
Fasting or	≥ 7.0	≥ 126
2-hour post-75g glucose load	≥ 11.1	≥ 200
Impaired glucose tolerance		
Fasting (if measured) and	< 7.0	<126
2-hour post-75g glucose load	≥7.8 and <11.1	≥140 and <200
Impaired fasting glucose		
Fasting and (if measured)	≥5.6 and <7.0	≥ 100 and <126
2-hour post-75g glucose load	<7.8	<140

1. Source: WHO (2006b).

1.5. Pathophysiology

Type I diabetes mellitus

In this case, insulin secretion is very low because of defective β-cells function. Untreated type I diabetes is characterized by hyperglycemia, hypertriglyceridemia, and episodes of severe ketoacidosis. The insulin /glucagon ratio can't increase; the liver is always gluconeogenic and ketogenic and can't properly buffer blood glucose levels. Hepatic gluconeogenesis is continuous; the liver contributes to hyperglycemia in the well-fed state (Guthrie and Guthrie, 2004). Accelerated gluconeogenesis, fueled by uncontrolled proteolysis in skeletal muscle, maintains the hyperglycemia even in the starved state. On the other hand, uncontrolled lipolysis in adipose tissue increases plasma fatty acid levels and ketone bodies production by liver. Ketoacidosis develops due to accumulation of ketone bodies and hydrogen ions (Harris and Crabb, 2006).

Fatty acid oxidation and ketogenesis cannot completely dispose fatty acids taken up by liver, and the excess is esterified and directed into VLDL synthesis. Hypertriacylglyceridemia results because VLDL and chylomicrons cannot be cleared

from the blood by lipoprotein lipase, whose expression is dependent upon insulin (Maghrani *et al*., 2004).

Type II diabetes mellitus

The individuals are resistant to insulin and have insufficient production of insulin to overcome the resistance. The majority of patients are obese, and although their insulin levels are often high, but they are not as high as those of nondiabetic. While the body still produces insulin, it is not enough to control glucose production by the liver or promote uptake of glucose by skeletal muscle (Kahan and Flier, 2000).

Type II diabetes is characterized by hyperglycemia, often with hypertriglyceridemia. The ketoacidosis rarely develops. Hyperglycemia results from both reasons. The normal increase in fructose 2, 6-bisphosphate and down-regulation of phosphoenolpyruvate carboxy kinase, do not occur in these patients (Harries and Crabb, 2006).

Hypertriacylglycerolemia is a characteristic and usually results from an increase in VLDL without hyperchylomicronemia; this may be due to hepatic synthesis of fatty acid and diversion of fatty acids reaching the liver into triacylglycerol and VLDL. Lipogenesis and gluconeogenesis should never occur, but in this disease they results from a state of mixed insulin resistance of the insulin signaling pathways controlling these processes. A defect in the insulin-signaling pathway controlling gluconeogenesis prevents suppression of hepatic glucose production in the presence of high insulin levels. A more responsive insulin-signaling pathway controlling fatty acid synthesis and esterification leads to over production of triacylglycerol (Harries and Crabb, 2006).

1.6. Complications

Diabetes mellitus and its complications are the major cause of morbidity and mortality in the United States (Deshpande, *et al*., 2008). Diabetes can affect many

different organ systems in the body, and over time can lead to serious complications. Complications from diabetes can be classified as microvascular or macrovascular. Microvasacular complications include nervous system damage (neuropathy), renal system damage (nephropathy), and eye damage (retinopathy) (ADA, 2006). Macrovascular complications include cardiovascular disease, stroke, and peripheral vascular disease. Peripheral vascular disease may lead to bruises or injuries that do not heal, gangrene, ultimately, amputation (Deshpande, *et al*., 2008). Figure 1 shows the prevalence of the most common diabetes complications among people with type 2 diabetes, Data from the 1999-2004 National Health and Nutrition Examination Surveys indicate that the prevalence of microvascular complications are much higher than the prevalence of macrovascular complications.

Heart Disease and stroke

Cardiovascular disease cases constitute up to 65% of all deaths in people with diabetes (Geiss, *et al*., 1995). Ischemic heart disease and stroke account for the greatest proportion of morbidity associated with diabetes. Mortality rates due to heart disease are 2-4 times higher among people with diabetes compared with those without diabetes. More than 60% of people with diabetes have high blood pressure or are being treated with medications for hypertension (Deshpande, *et al*., 2008).

Peripheral Arterial disease

This disease is also referred to as peripheral vascular disease, caused by the narrowing of blood vessels that carry blood to the arms, legs, stomach, and kidneys. In people with diabetes, the risk factor peripheral Arterial disease increases by age, duration of diabetes, and by the presence of neuropathy (King, *et al*., 2005).

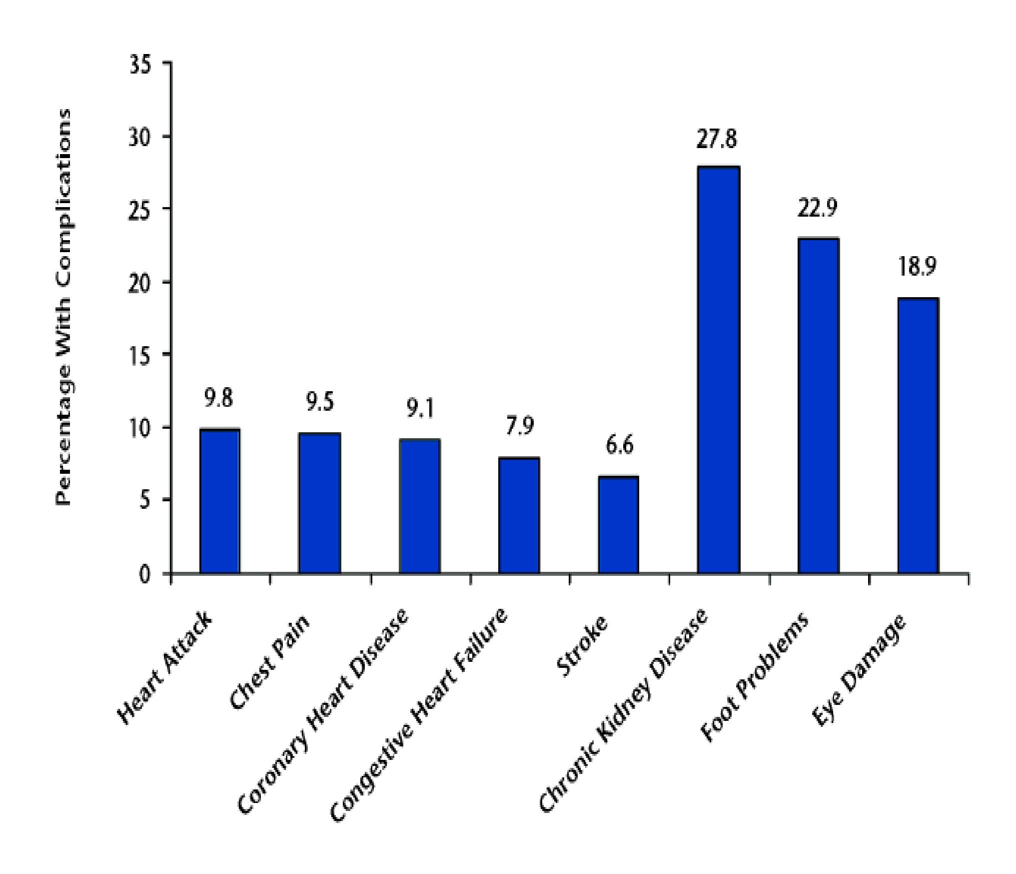


Figure1. Prevalence of diabetes-related complications among people with diabetes. Natienal Health and Nutrition Examination Surveys, 1999-2004 (Deshpande, *et al*., 2008).

Nephropathy (Renal Disease)

Diabetic nephropathy is defined as persistent proteinuria (more than 500 mg of protein or 300 mg of albumin) in patients without urinary tract infection or other diseases causing the proteinuria. In patients with type I diabetes, development of clinical nephropathy is a relatively late event; however, in patients with type II diabetes, diabetic proteinuria may be present (Bojestig, *et al*., 1994).

Retinopathy (Blindness)

Retinopathy is associated with prolonged hyperglycemia, it is slow to develop, and there is some evidence that it can begin to develop as early as 7 years before clinical diagnosis of type 2 diabetes (Harris and Leininger, 1993). The prevalence of visual impairment among people with diabetes increases with age. As much as 90% of blindness due to retinopathy among people with diabetes may be preventable if detected and treated early. Annual dilated eye examinations are recommended for all patients with diabetes (ADA, 2006).

1.7 Management

The main goal of treatment of diabetes mellitus is to maintain blood glucose level in the normal range or as close to normal and to prevent long-term complications. Treatment of diabetes is mainly based on appropriate plan for meals and physical activity, as well as medication if necessary. Insulin therapy is necessary in type I diabetes mellitus. Nutritional assessment and physical activity are recommended in the treatment of type I diabetic patients in order to avoid or to control diabetes complications. However, treatment of type II diabetes patients started by nutritional therapy and physical activity. Medical intervention must be done if blood glucose concentration is not controlled by nutritional therapy and physical activity (ADA, 2002).

Type II diabetes patients are characterized by over weight and abdominal fat distribution that is highly related to insulin resistance, so weight loss is recommended (Anderson, 2006). American Diabetes Association (2008) recommended a diet, which conforms to the criteria of healthful diet, with 55-65% carbohydrate, 15-20% protein, ≤30% fat of total caloric intake, 25g/1000kcal fiber and low cholesterol diet (<200mg/dl), saturated fatty acids< 7% of total energy, minimal intake of trans fatty acids. In addition, two or more serving of fish per week.

1.8. Experimental Diabetes

Chemically induced diabetes is the most common method used to induce diabetes experimentally; one of the best recommended models to study the impact of diabetes on rats and mice is the induction of diabetes by streptozotocin (STZ) or alloxan (Ogasawara, *et al*., 2008). Both are cytotoxic glucose analogues. Although their cytotoxicity is achieved via different pathways, their mechanisms of beta cell selective action are identical (Lenzen, 2008). STZ is an N-nitroso derivative of D-glucoseamine and is usually isolated from certain fungi types (Bonner-Weir, *et al*., 1981).

STZ enters the beta cell via a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of STZ than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD^- and ATP. Enhanced ATP dephosphorylation after STZ treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, STZ liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the STZ action, β cells undergo the destruction by necrosis (Szkudelski, 2001).

Previous studies categorized STZ induced diabetes in rats broadly into type I (STZ dose >50mg/ Kg body weight) and type II (STZ dose <50 mg/ Kg body weight) diabetes (Ugochukwu, *et al*., 2004). Some studies showed that 32.25 mg/Kg body weight injection of STZ produced type II diabetes (Kanarek and Ho, 1984).

The effectiveness of these diabetogenic chemicals is highly dependent on the age and species of the recipient (Junod, *et al*., 1969). The effects of STZ appear within hours of administration in Sprague Dawley neonatal rats and produce symptoms quite similar to human diabetes (Rabinowitz and Craig, 1989).

2. Oxidative Stress

2.1. Introduction

Oxidative stress was first defined in 1985, as a situation in which increased level of free radicals and reactive oxygen species (ROS) generation overwhelms the antioxidant defense system capacity (Sakano, *et al*., 2009). Oxidative stress can also be defined as an imbalance between ROS and the antioxidative defense in the body (Montuschi, *et al.,* 2004).

The antioxidant defense system consists of enzymatic and non-enzymatic antioxidants. Principal antioxidant enzymes include superoxide dismutase, glutathione peroxidase, and catalase which function as cellular protection against oxidation. On the other hand, the non-enzymatic antioxidants include glutathione (GSH), uric acid, bilirubin, coenzyme Q10 (ubiquinone) and dietary antioxidants such as β-carotene, vitamin C, and vitamin E (Powers and Jackson, 2008).

Free radicals are atoms or molecules that have unpaired electrons on the outer orbital, which make them unstable and highly reactive. However, the term free radical is not ideal to describe the group of reactive pathogenic species, because some of them do

not have unpaired electrons on the outer orbital, so the use of ROS term is considered to be more appropriate (Cavalcante and Bruin, 2009).

Reactive oxygen species such as superoxide anion (O_2^-) and hydroxyl radicals ($OH^·$) are continuously generated as a result of oxygen metabolic processes (Sakano, *et al*., 2009). Endogenously ROS are mainly generated within the mitochondrial respiratory chain, or through activity of NADPH oxidases (Sauer and Wartenberg, 2008). Exogenous sources are environmental toxins and air pollutants such as ozone, nitric dioxide, sulfur dioxide and cigarette smoking (Montuschi, *et al*., 2004). The generation ROS can attack lipids, protein, and DNA resulting in oxidative damage to these molecules.

2.2 Oxidative Stress and Human Disease

Oxidative stress is recognized to have a role in a wide variety of human diseases, including cancer, cardiovascular, pulmonary, neurological, renal, liver diseases and even the normal aging process (Montuschi, *et al*., 2004). Although, the relation between increased oxidative stress and disease does not necessarily imply a causative link, the fact that oxidative stress biomarker levels are usually increased in patients with asthma, diabetes, alcoholic liver, cardiovascular disease and chronic obstructive pulmonary disease suggests a causative role of oxidative stress at least in these diseases (Sakano, *et al.,*2008).

It has been found that levels of 15-F_{2t}-Isoprostane; the most sensitive and specific biomarker of lipid peroxidation in vascular disorders (Cracowskia and Ormezzanob, 2004), was not only elevated under conditions of ischemia/ reperfusion and atherosclerosis, but also in patients with cardiovascular risk, such as smoker, hypercholesterolemic, and obese (Sauer and Wartenberg, 2008). Oxidative stress

biomarkers were also found to be elevated in both type I and type II diabetes by three folds compared to healthy subjects (Kaviarasan, *et al.,* 2009).

2.3 Oxidative Stress and Diabetes Mellitus

Diabetes mellitus is associated with increased formation of free radicals and decrease in antioxidant potential. This leads to oxidative damage of cell components such as protein, lipids, and nucleic acids in both insulin dependent and non-insulin dependent diabetes (Nazirog and Butterworth, 2005).

Oxidative stress is increased and may accelerate the development of microvascular complications including retinopathy, nephropathy, and neuropathy as a result of hyperglycemia and diabetes (Scott and King, 2004). The increase of oxidative stress in patients who have diabetes and poor glycemic control or insulin resistance is most likely a result of abnormal metabolism such as hyperglycemia, dyslipidemia, and elevated of free fatty acids (FFAs) (Evans, *et al.,*2003; Brownlee, 2001).

In hyperglycemia, increase of oxidants can arise from several processes. For example, gluco-oxidants and advanced glycated end products are created by non-enzymatic reactions thereby adding to the increased levels of oxidants (Will*, et al.,*1999 ;Baynes and Thorpe, 1999). These processes can also form oxidized low-density lipoprotein and nitrotyrosine both extracellularly and intracellularly. It has been suggested that the production of superoxide due to hyperglycemia can also be derived from glycolysis and mitochondrial oxidative phosphorylation (Brownlee, 2001). Elevated intracellular glucose can alter the redox balance by increasing flux through the aldose reductase pathway, inhibition of the electron transport chain in the mitochondria, activation of oxidase, and alteration of NADPH/NADP ratios (Brownlee, 2001; Dunlop, 2000). Furthermore, byproducts of these processes can cause activation of certain signaling cascades such as protein kinase C, which can activate oxidases to increase

superoxide production (Inoguchi, *et al.,* 2000). Thus, metabolism of high concentrations of glucose can activate NADPH oxidase in vasculare cells independent of mitochondrial metabolism to increase oxidants (Inoguchi*, et al*., 2000).

Elevated free fatty acids levels can increase oxidant production by β oxidative phosphorylation via mitochondrial metabolism (Evans, *et al.,* 2003; Boden and shulman, 2002). It has been documented that malondialdehyede levels and NF-κB expression are increased in insulin-resistant states without hyperglycemia in vascular tissues as well as in muscle and adipose tissues (Itani, *et al.,* 2002). Furthermore, it was demonstrated that glutathione levels in the plasma can be decreased by free fatty acids infusion (Paolisso, *et al*., 1996).

3. Olive Oil

3.1. Introduction

Olive oil is an important component in the Mediterranean diet and considered the main source of fat in the Mediterranean area (Waterman and Lockwood*,* 2007). Olive oil contributes about 4% of total vegetable oil production: its world production is around 2,000,000 tons/year and the Mediterranean countries contribute more than 95% of the world production (FAOSTAT database, 2000).

Olive oil is widely used in Jordan, due to abundance of olive trees throughout the country. According to Department of Statistics/ Agricultural census 2007, olive trees cover around 601,401dunoms representing 74% of all planted area in Jordan. Depending on the Department of Statistics/ Food Balance Sheet 2009, the average annual consumption of olive oil was 2.8 kg/year in 2007 and reached 3.4 kg/year in 2009. The increased consumption of olive oil may be due to its abundance in the Kingdom, or due to the belief that it has a beneficial role in health or due to both factors.

3.2. Composition of Olive Oil

Olive oil is a functional food which besides having a high level of MUFA, the oleic acid, contains multiple minor components with biological properties. The content of the minor components of an olive oil varies, depending on the cultivar, climate, ripeness of the olives at harvesting, and the processing system employed (Gimeno, *et al.,* 2002).

Virgin olive oil are those obtained from the fruit of the olive oil tree solely by mechanical or other physical means under conditions that do not lead to alteration in the oil. They have not undergone any treatment other than washing, decantation centrifugation or filtration (Visiol and Galli, 2002).

The chemical composition of virgin olive oil is divided into major components, which include glycerols, representing more than 98% of total oil weight, and minor components. These components, which are presented in very low amounts (about 2% of oil weight), include more than 230 chemical compounds, such as a liphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds and antioxidants (carotenoids, lipophilic, and hydrophilic phenolic compounds) (Servilli, *et al.,*2004). Some of these minor components of olive oil, mainly the hydrophilic phenols, are removed during refining process. As a consequence, these compounds are specific to virgin olive oil (Covas, *et al.,* 2006b).

Extra virgin olive oil is virgin olive oil with free fatty acidity, expressed as g of oleic acid/ 100g of olive oil, less than 0.8g.Virgin olive oil with an acidity less than or equal 2 g (Jordanian standard).

3.3. Effect of Olive Oil on Health

Epidemiological studies have clearly shown that the habitual consumption of olive oil, as a principle fat source in diet, is associated with a low incidence of coronary

heart disease, certain types of cancers, modulation of immune and inflammatory responses. It seems that this production is due to diverse combinations of biological effects including antioxidant (Visioli, *et al*., 2006), anti-inflammatory (Fito, *et al*., 2005), vasodilatation (Visiolli and Galli, 2002), and anti-platelet aggregation properties (Fito, *et al.,* 2005). These effects attributed to the olive oil composition of high level of MUFA and minor components with biological properties (Tur, 2004). This minor components are classified into two types, the unsaponifiable (non-polar) and soluble (polar) fraction, which includes the phenolic compounds (Owen, *et al*., 2000).

In experimental studies, polyphenol components of olive oil have bioactive properties, including anti-inflammatory, antioxidant, antiarrhythmic, and vasodilatory effect (Covas, *et al*., 2006a). The insoluble fraction of virgin olive oil has been shown to inhibit both LDL receptor-related protein expression and activity (Perona and Avella, 2006). Cabello-Moruno, *et al*. (2007) found triacylglycerol concentration of triacylglycerol-rich lipoproteins (TRL) and their particle size were higher, and the number of (TRL) particles was lower after virgin olive oil, with a high content of non-polar phenols, than after refined olive oil.

Recent studies have supported the proposed mechanisms by which virgin olive oil can exert its beneficial effects on cardiovascular risk (Perez-Jimenez, *et al.,*2007; Carluccio, *et al.,* 2007), including: 1) improvement of lipid profile, through a decrease in total cholesterol and LDL-C and increase of the HDL-C/ cholesterol ratio (Estruch, *et al.,* 2006; Vincent-Baudry, *et al.,*2005); 2) reduction of LDL susceptibility to oxidation and amelioration of oxidative vascular damage (Estruch, *et al*., 2006; Ruano, *et al*.,2005); 3) improved endothelial function (Ruano, *et al*.,2005; Fuentes, *et al*.,2008); 4) improved blood pressure control (Bondia-Pons, *et al*.,2007).

Many studies have suggested the beneficial effect of olive oil on blood when compared with high oleic sunflower oil. In hypertensive women, olive oil consumption in the regular diet for four weeks has shown a decrease in both systolic and diastolic pressures when compared to high oleic sunflower oil (Stark and Madar, 2002). Similarly, in hypertensive rats maximum contraction of aortic ring was significantly lower when olive oil was consumed compared to high oleic sunflower oil. These results suggested that other components of olive oil rather than oleic acid are thought to be responsible for vascular effect (Stark and Madar, 2002).

The data from EPIC (Romaguera *et al.*, 2009) show lower waist girth with increasing adherence to the Mediterranean diet, also support a beneficial role on metabolic syndrome. Results of a study in overweight, insulin patients also suggest that, comparing with a low-fat diet, a high-virgin olive oil diet prevents the redistribution of body fat from peripheral to visceral adipose tissue without affecting total body weight (Paniagua, *et al.*, 2007a).

In countries where the population adheres to the Mediterranean diet, such as Spain, Greece, and Italy, which olive oil is the principal source of fat, rates of cancer incidence are lower than in northern European countries. Furthermore, results of case control and cohort studies suggest that MUFA intake including olive oil is associated with a reduction in cancer risk [mainly breast, colorectal, and prostate cancers] (López-Miranda, *et al.*,2010).

3.4. Effect of Olive Oil on Diabetes Mellitus

Olive oil has been found to have a positive effect on glucose tolerance and insulin sensitivity. A study investigated the influence of dietary fat quantity and composition on glucose tolerance and insulin sensitivity in rats, replacing saturated fatty

acid with MUFA does not impair glucose tolerance or insulin sensitivity (Alsaif and Duwaihy, 2004).

Diets high in saturated fatty acid (SAF) consistently impair both insulin sensitivity and blood lipids, while substituting CHO or MUFA for SFA reverts these abnormalities (Riccardi, *et al.,*2004). Post-prandial lipemia and glucose homeostasis are also improved after meals containing MUFA from olive oil compared to meals rich in SAF (Paniagua, *et al.,* 2007b; Lopez, *et al*., 2008). On the other hand, MUFAs post-prandially buffered β cell hyperactivity and insulin intolerance relative to SFAs in subjects with high fasting triglyceride concentrations (López-Miranda, *et al*., 2010).

Generally high MUFA diets had more favorable effects on proatherogenic alterations associated with the diabetic status ,such as dyslipidemia, post-prandial lipemia, small LDL, lipoprotein oxidation, inflammation, thrombosis, endothelial dysfunction (Ros, 2003). The administration of olive oil to normal and diabetic rats showed a better profile in the lipid as well as decrease in the concentration of lipid hydrperoxidase (Alhazza, 2007).

Jemai, *et al*., (2009) studied the effect of hydoxytyrosol and oleuropein in diabetic rats and they found significant decrease in the serum glucose and cholesterol levels and restored the antioxidant perturbations, suggesting the antidiabetic effect of oleuropein and hydroxytyrosol might be their antioxidant activities restraining the oxidative stress which is widely associated with diabetes pathologies and complication.

Hydroxytyrosol decreased triglycerides; total cholesterol and higher HDL in serum. Olive mill waste polyphenols and hydroxytrosol are efficient in inhibiting hyperglycemia and oxidative stress induced by diabetes and suggests that administration of hydroxytyrosol may be helpful in preventing diabetic complication associated with

oxidative stress (Hamden, *et al*., 2009). Long-term regular intake of virgin olive oil consumption protected the endogenous antioxidant system (Valls, *et al.,* 2010).

Materials and Methods

1. Olive Oil Preparation and Characterization

Virgin olive oil used in the present experiment was purchased from the local market during the season, 2010 (AL-Mishkat company, Jordan) .The peroxide value and acidity % were tested according to AOAC, (1995) to ensure oil conformation with the Jordanian standards .The quantity of oil was divided into three equal portions, first portion was left as a virgin olive oil as purchased, and kept refrigerated at $4C^{\circ}$.

The other two portions were placed in clean and dry beakers 500ml in the oven (Memmert, Germany) at $60\pm2C^{\circ}$ with active air circulation. This method was conducted to evaluate the effect of oxidation during the accelerated storage conditions which is known as Schaal oven test (Velasco and Dobarganes, 2002), and simulate the real storage conditions in a market or a home place. During the storage, samples were taken periodically (daily) for analysis and the progress of oxidation was followed by peroxide value, in order to obtain 1.5x and 2.0x of the maximum recommended value (x) according to the Jordanian standards, which equals 20, (1.5x P.V=30 meqO_2/kg oil and 2.0x P.V= 40 meqO_2/kg oil). At each point of peroxide value, a quantity of the oil was then taken and kept refrigerated at $4C^{\circ}$ until used.

1.1. Chemical Analysis of Olive Oil

1.1.1. Peroxide Value

Peroxide value is one of the chemical quality determinations of olive oil; it was carried out in duplicate for each replicate according to the methods described by AOAC, (1995).Where:

5.0g of olive oil sample was placed in 250 ml Erlenmeyer flask, the sample was dissolved in a 30 ml of 3:2 acetic acid: chloroform solution. Subsequently, 0.5 ml of

saturated, freshly prepared, potassium iodide (KI) solution was added, the mixture was shaken vigorously for 1 min. Then, 30ml distilled water and 1 ml of starch solution 1% (w/v) were added. Finally, the mixture was slowly titrated at 0.1M of Sodium thiosulfate ($Na_2S_2O_3$) with vigorous shaking until the blue color just disappeared.

The peroxide value was calculated according to the following formula and the results were expressed as milliequivalents of oxygen per kilogram of oil ($meqO_2$ / Kg oil):

$$P.V = \frac{(Vs - V_b) * 1000 * M}{\text{Weight of sample (g)}}$$

Where:

V_s = volume of sodium thiosulfate consumed by sample in ml.

V_b = volume of sodium thiosulfate consumed by blank in ml.

M = molarity of sodium thiosulfate.

1.1.2. Acidity

Acidity is also considered a chemical quality determination of olive oil. The procedure was performed according to an AOAC, (1995) as follows:

5.0g of olive oil sample was placed in a 250 ml Erlenmeyer flask, the sample was dissolved in a 50 ml ethanol: diethyl ether mixture 1:1, few drops of phenol phthalin indicator 1% was added. Then, the mixture was slowly titrated with sodium hydroxide solution 0.1M until the color turned faint pink. Acidity was calculated according to the following formula and expressed as % oleic acid:

$$\text{Acidity (\% oleic acid)} = \frac{(Vs - V_b) * M * 0.282 * 100}{\text{Weight of sample (g)}}$$

Where:

V_s = volume of sodium hydroxide consumed by each sample in ml.

V_b = volume of sodium hydroxide consumed by blank in ml.

M = molarity of sodium hydroxide.

282 = molecular weight of oleic acid.

1.1.3. Total Antioxidant Capacity

Total antioxidant capacity (TAC) of olive oil was measured by the total antioxidant status (TAS) Kit that was purchased from RANDOX laboratories (Ltd), United Kingdom. TAC of olive oil was measured manually using a Spectrophotometr (Spectro UV – V, Double PC, Labomed. Inc., USA). In this test, ABTS® (2, 2' –Azino – di-[3- ethyl enzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and H_2O_2 in order to produce the radical cation $ABTS^{\cdot+®}$. This has a relatively stable blue–green colour, which is measured at 600nm. Antioxidants in the added sample cause suppression of this colour production to a degree which is proportional to their concentration. The principle of the test can be summarized by these equations:

$HX – Fe^{III} + H_2O_2 \longrightarrow X\text{-}[Fe^{IV} = O] + H_2O$

$ABTS^{®} + X\text{-}[Fe^{IV} = O] \longrightarrow ABTS^{®\cdot+} + HX – Fe^{III}$

$HX – Fe^{III}$ = Metmyoglobin

X -[Fe^{IV} = 0] = Ferrylmyoglobin

ABTS® = 2, 2′–Azino – di - [3- ethylbenzthiazoline sulphonate]

Reagent Composition:

R_1: Buffer (Phosphate Buffered Saline: 80 mmol / l, pH 7.4)

R_2: Chromogen (Metmyoglobin: 6.1 µmol / 1; ABTS®: 610 µmol/1)

R_3: Substrate (Hydrogen Peroxide (in stabilized form): 250µmol /1)

CAL: Standard (6- hydroxy – 2,5,7,8 – tetramethylchroman -2- carboxylic acid: 2.08mmol/1).

The procedure was done under controlled temperature (37C°), wavelength 600nm and1cm light path of cuvette.

The following table summarizes the procedure:

	Reagent blank	Standard	Sample
DDH_2O	20 µl	----	----
Standard	----	20 µl	----
Sample	----	----	20 µl
Chromogen (R_2)	1 ml	1 ml	1 ml

Each reagent was mixed well, incubated to bring to temperature and read initial absorbance (A_1). Then, the substrate hydrogen peroxide was added for each reagent with a concentration of 200µl, then mixed and started timer simultaneously and read absorbance after exactly 3 minutes (A_2).The change of absorbance was measured for each reagent ($\Delta A = A_2 - A_1$). Finally, total antioxidant status of olive oil (mmol /1) was calculated by the following equations:

Factor = concentration of standard / (ΔA blank - ΔA standard)

Mmol /1 = Factor * (ΔA blank – ΔA sample).

3. Preparation of Experimental Diet Mixtures

Three diet mixtures were prepared according to Reeves (1997) that have the same amount of protein, vitamins, and minerals per KJ, and contain 4% of either virgin olive oil (as base line of P.V), or stored olive oil preparations(1.5x P.V and 2.0x P.V). Three other diet mixtures were prepared in the same manner but contain 8% of virgin olive oil or stored olive oil preparations. The recommended fat content in normal diets of rats was 4%. The added amount of fat was put at the expense of carbohydrate energy in the diet mixture. It was formulated by mixing specific amounts of virgin olive oil, stored olive oil preparation (1.5x P.V and 2.0x P.V), corn starch, casein, fibers ,water soluble vitamins, fat soluble vitamins, DL-methionine, and choline bitartrate.

Table 2: Ingredient composition of experimental diets

Components	Diet mixtures (g/kg)	
	4% Olive oil	8% Olive oil
Casein	140	140
Corn starch	720.2	680.2
Olive oil	36	76
Water soluble vitamin mix[1]	10	10
Fat soluble vitamin mix[2]	4(5ml)	4(5ml)
Mineral mix[3]	35	35
DL-Methionin	2.3	2.3
Choline bitartrate	2.5	2.5
Fibers	50	50

(1)1kg water soluble vitamin mix composed of the following: 3.0g nicotinic acid, 1.6g calcium pantothenate, 0.7g pyridoxine-HCL, 0.6g thiamin-HCL, 0.6g riboflavin, 0.2g folic acid, 0.02g biotin, 2.5mg cyanocobalamin (vit B12), and 990.8g powdered sucrose (Adapted from Reeves, 1997).

(2) Fat soluble vitamin mix composed of the following: 0.088g vitamin A(retinyl palmitate), 3g tocopherol acetate, 0.03g vitK_1(phylloquinone), and 0.001g vitD_2 (Ergobcalciferol) all these compounds was dissolved in 200ml of oil, to make 1kg of diet need 5ml from this mixture which equal 5g .

(3)1kg of mineral mix composed of the following: 357g $CaCO_3$, 74g NaCl, 159.8g K_2HPO_4, 46.6gK_2SO_4, 28g $C_6H_5K_3O_7$., 24g MgO, 6.06g Ferric Citrate, 3.78g $ZnSO_4$, 1.22g $MgSO_4.4H_2O$, 0.68g $CuSO_4$, 0.01g KI, 0.0102g Na_2O_4Se , 0.008g Ammonium Paramolybdate,4hydrate, 1.45g Sodiummeta-Silicate, 9 hydrate, 0.28g $CrK(SO_4)_2.12\ H_2O$, 0.0815g H_3BO_3, 0.0635g NaF, 0.0174g LiCl, 0.0087g VCl_3,and 296.9g powdered sucrose.

The ingredient composition of 4% and 8% olive oil diet mixtures is shown in table (2). The energy content of the diet were 1.65 and 1.74 mega joules / 100g (MJ / 100g) for respective fat contents (4% and 8%) These diets provided 9.1% and 17.4% of energy from fat respectively and contained 14% protein. Experimental diets were freshly prepared twice a week and stored refrigerated at $4C^{\circ}$.

4. Preparation of the Experimental Animals

Forty–eight male Sprague–Dawley rats, weighing approximately 218g were purchased from the Faculty of Medicine, University of Jordan. Rats were weighed and individually housed in plastic cages with a stainless steel wire floor and front (North Kent plastic cages, England). They were fed *ad libitum* on stock and tap water for two weeks prior to start of the experiment for acclimatization. Environmental conditions were under control with $20 \pm 2C^{\circ}$ and 12 hours light–dark cycle. Diets were given in glass jars and water was provided in glass bottles with rubber stoppers.

5. Induction of Diabetes

After acclimatization, the forty–eight rats were subjected to 12 hours fast. Diabetes was partially induced in each rat by a single intraperitoneal streptozotocin (Sigma Chemicals Co., Mo, U.S.A) injection (32.25 mg/ml freshly dissolved in 0.05M citrate buffer, pH 4.5 at a dose of 32.25 mg/kg) (Kanarek and HO, 1984; Yamada *et al* .,2002). After 36 hours, diabetes was checked by testing glucosuria of the rats using glucose urine strips (Glkotest, Rocho Germany), and by observing polydipsia and polyuria.

6. Experimental Design

Rats were divided into six groups, eight rats for each group, having the same average of body weights and standard deviations. Figure (1) shows the experimental arrangement of rats in the study. Experimental feeding continued for five weeks with free access to

water and food. Animal weights and food intake were monitored once a week, water intake was measured daily.

At the end of experiment periods, rats were starved for 12 hours; anesthetized by chloroform, and then blood was drawn from right ventricle of the heart using a medicinal syringe and transferred to plain tubes, centrifuged at 3000 rpm for 20 minutes, to obtain serum. Samples of the serum were stored frozen at -18C° for analysis. Livers were excised, cleaned and weighed and stored frozen at -18C°.

7. Biochemical Tests

Serum total antioxidant capacity (TAC), glucose, and lipid profile were analyzed in medical laboratories of Khalidi Medical Center (Amman, Jordan). TAC test was carried out on Hitachi 911 analyzer (Tokyo, Japan), and serum glucose and lipid profile were carried out on (COBAS INTEGRA 400 plus), an automated clinical chemistry analyzer. Before performing the tests, calibration of the analyzer for TAC, glucose, triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL–C) and high-density lipoprotein cholesterol (HDL–C) were done according to the manufacture instructions. The analytical procedures were performed as follows:

7.1 Total Antioxidant Capacity

Total Antioxidant Capacity (TAC) was measured by using TAC kit which was purchased from Randox Laboratories Ltd. (Crumlin, United Kingdom), TAC test was carried out on automated chemistry analyzer Hitachi 911(Tokyo, Japan). Test principle: ABTS® (2,2′- Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and H_2O_2 in order to produce the radical cation $ABTS^{\cdot+}$®.

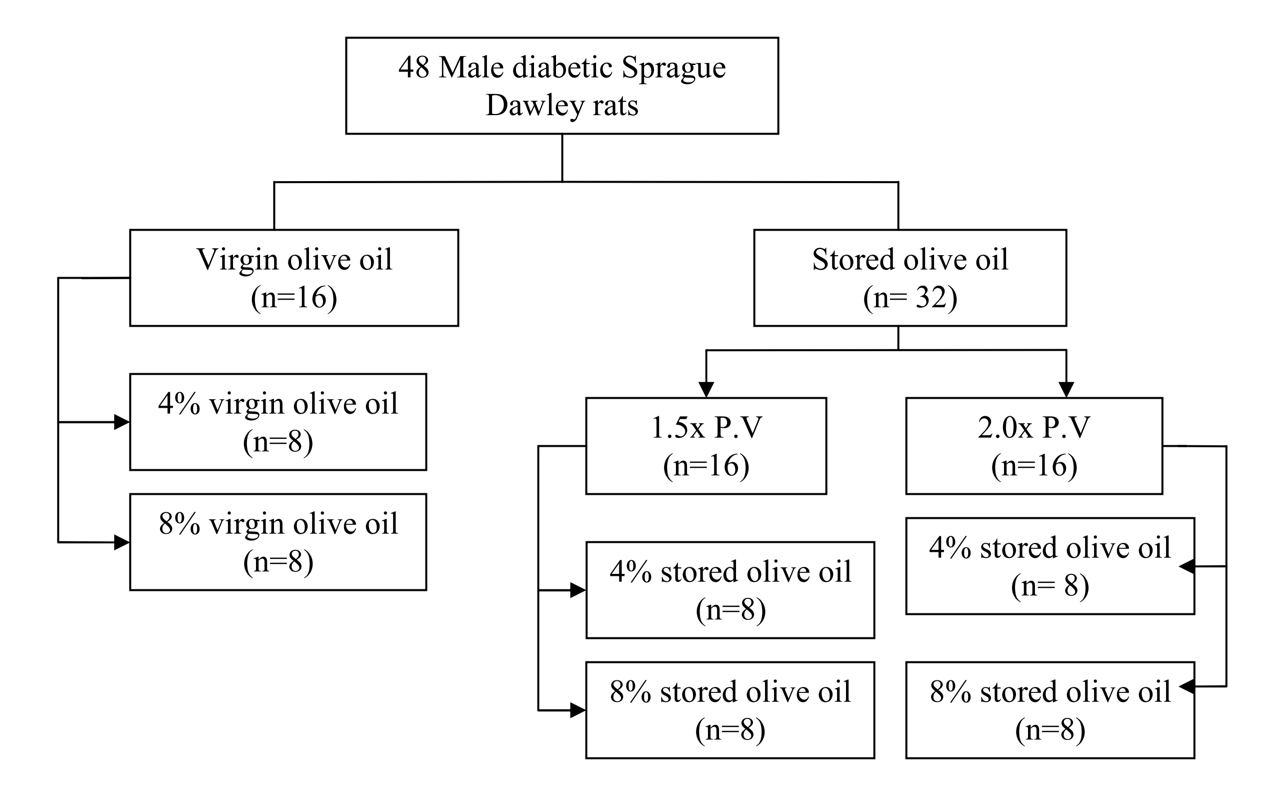


Figure 2: Experimental arrangements of rats in the study..

x: maximum peroxide value (P.V) allowed according to the Jordanian standards that equals $20meqO_2/kg$ olive oil.

This has a relatively stable blue–green color, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a proportional degree to their concentration.

$HX – Fe^{III} + H_2O_2 \longrightarrow X\text{-}[Fe^{IV} = O] + H_2O$

$ABTS^{®} + X\text{-}[Fe^{IV} = O] \longrightarrow ABTS^{®\cdot+} + HX – Fe^{III}$

$HX\text{-}Fe^{III}$ = Metmyoglobin

$X\text{-}[Fe^{IV}$ =0] = Ferrylmyoglobin

$ABTS^{®}$ =2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]

$ABTS^{®}$ is a registered trademark of Boehringer Mannheim.

Reagent–working solutions:

R1 Buffer (Phosphate Buffered Saline: 80 mmol/l, pH 7.4)

R2 Chromogen (Metmyoglobin: 6.1 µmol/l; $ABTS^{®}$:610 µmol/l)

R3 Substrate (Hydrogen peroxide (in stabilized form): 250 µmol/l).

CAL. Standard(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid: 2.08 mmol/l).

For confirmation of accuracy and reproducibility, a Randox TAC control was used.

7.2 Glucose

Biochemical test of serum glucose was performed using COBAS INTEGRA Glucose HK Gen.3 (GLUC3). The test principle was based on enzymatic reference method with hexokinase. Hexokinase (HK) catalyzes the phosphorylation of glucose by ATP to form glucose-6-phosphate and adenosine diphosphate (ADP).

To follow the reaction, a second enzyme, glucose-6-phoshate dehydrogenase (G6PDH) is used to catalyze oxidation of glucose-6phosphate by nicotinamide adenine dinucleotide phosphate($NADP^+$) to form NADPH.

D-glucose +ATP $\xrightarrow{HK}$ D-glucose-6-phosphate+ ADP.

D-glucose-6-phosphate+$NADP^+$ $\xrightarrow{G6PDH}$ D-6-Phosphogluconate+ $NADPH+H^+$

The concentration of the NADPH formed was directly proportional to the glucose concentration. It was determined by measuring the increase in absorbance at 340nm (Trinder, 1969).

7.3 Triglycerides .

Serum triglycerides (TG) content was measured using COBAS INTEGRA Triglycerides (TRIGL). The principle of test depends on some enzymatic reactions: lipoprotein lipase, glycerol kinase (GK) and glycerol-3-phosphate oxidase (GPO).

Triglycerides + $3H_2O$ $\xrightarrow{\text{Lipoprotien lipase}}$ glycerol + 3 RCOOH

Glycerol + ATP $\xrightarrow{\text{GK} \quad Mg^{2+}}$ glycerol-3-phosphate +ADP

Glycerol-3-phosphate+O_2 $\xrightarrow{\text{GPO}}$ dihydroxyacetone phosphate + H_2O_2

H_2O_2+ 4-aminophenazone + 4-chlorophenol $\xrightarrow{\text{POD}}$ 4-(p-benzoquinone-monoimino) – phenazone + 2 H_2O + HCL.

The color intensity of the red dye stuff formed was directly proportional with the triglyceride concentration, and was determined photometrically by measuring the absorbance at 505 nm (Fossati and Prencip, 1982).

7.4 Total Cholesterol

Serum total cholesterol (TC) was performed using COBAS INTEGRA Cholesterol Gen.2 cassette (CHOL2). TC was measured by enzymatic calorimetric method. Cholesterol esters were cleaved by the action of cholesterol esterase (CE) to yield free cholesterol and fatty acids. Cholesterol oxidase (CHOD) then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine to form a red quinone-imine dye.

Cholesterol esters + H_2O $\xrightarrow{\text{CE}}$ Cholesterol + RCOOH

Cholesterol + O_2 $\xrightarrow{\text{CHOD}}$ cholest-4-en-3-one + H_2O_2

$2\ H_2O_2$ + 4-AAP+ phenol $\xrightarrow{\text{peroxidase}}$ quinone-imine dye + $4H_2O$

The formed color intensity of the dye was directly proportional with the cholesterol concentration. It was determined by measuring the increase in absorbance at 512 nm (Siedal, *et al*., 1983).

7.5 High Density Lipoprotein-Cholesterol

Biochemical test of serum high-density lipoprotein cholesterol (HDL-C) was performed using COBAS INTEGRA HDL-cholesterol plus 3rd generation (HDLC3), which is designed to the direct specific determination of HDL-cholesterol in the presence of LDL, VLDL, and chylomicrons. No sample pretreatment step was required. HDL-C was measured by homogeneous enzymatic colorimetric assay. In the presence of magnesium ions and dextran sulfate, water-soluble complexes with LDL, VLDL, and chylomicrons were formed which are resistant to polyethylene glycol (PEG)-modified enzymes.

The cholesterol concentration of HDL was determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approximately 40%). Cholesterol esters were broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidease to 4-cholestenone and hydrogen peroxide.

HDL-cholesterol esters + H_2O $\xrightarrow{\text{PEG + cholesterol esterase}}$ HDL-C + RCOOH

HDL-C + O_2 $\xrightarrow{\text{PEG + cholesterol oxidase}}$ 4-cholestenone +H_2O_2

$2H_2O_2$ + 4-aminoantipyrine HSDA + H^+ + H_2O $\xrightarrow{\text{Peroxidase}}$ purple blue pigment + 5 H_2O

The color intensity of the blue quinoneimine dye formed is directly proportional to the HDL-C concentration. It was determined by measuring the increase in absorbance at 583 nm (Sugiuchi, *et al*., 1995).

7.6 Low Density Lipoprotein-Cholesterol.

Biochemical test of serum low-density lipoprotein cholesterol (LDL-C) was performed using COBAS INTEGRA LDL-Cholesterol plus 2^{nd} generation (LDL-C). The test principle was based on homogeneous enzymatic colorimetric assay. This automated method for the direct determination of LDL-C takes advantage of the selective micellary solubilization of LDL-C by a nonionic detergent and the interaction of sugar compound and lipoproteins (VLDL and chylomicrons). When a detergent is included in the enzymatic method for cholesterol determination (cholesterol esterase, cholesterol oxidase coupling reaction), the relative reactivities of cholesterol in the lipoprotein fractions increase in this order: HDL $<$ chylomicrons $<$ VLDL $<$ LDL. In the presence of Mg^{++} a sugar compound markedly reduces the enzymatic reaction of the cholesterol measurement in VLDL and chylomicrons.

The combination of a sugar compound with detergent enables the selective determination of LDL-C in serum. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to 4-cholestenone and hydrogen peroxide.

$$\text{LDL-C ester} + H_2O \xrightarrow{\text{detergent cholesterol esterase}} \text{cholesterol} + RCOOH$$

(Selective micellary solubilization)

$$\text{LDL-C} + O_2 \xrightarrow{\text{cholesterol oxidase}} \Delta\text{-4cholestenone} + H_2O_2$$

$$2\ H_2O_2 + \text{4-aminoantipyrine} + \text{HSDA} + H^+ + H_2O \xrightarrow{\text{Peroxidase}} \text{purple blue pigment} + 5\ H_2O$$

The color intensity of the blue quinoneimine dye formed is directly proportional to the LDL-C concentration. It was determined by measuring the increase in absorbance at 583 nm (Sugiuchi, *et al*., 1998).

8. Statistical Analysis

Statistical analysis of the measured and calculated data was performed using the statistical analysis system (SAS package, version 9).Values for all data were presented as mean± standard error of mean (SEM). Analysis of variance (two ways ANOVA) with LSD test was used to determine any significant differences between the mean at a significant level of 5 % ($P<0.05$). Pearson's correlation was performed on selected measured variables to express any relationship between them.

Results

1. Chemical Analysis of Olive Oil.

Table 3 shows peroxide value (P.V), acidity, and total antioxidant capacity (TAC)of virgin olive oil and stored olive oil preparations (1.5x P.V and 2.0x P.V). The P.V were 10, 30 and 40 meqO_2/kg oil for virgin olive oil and stored olive oil preparations 1.5x P.V and 2.0x P.V, respectively .The acidity of virgin olive and its stored preparations (1.5x P.V and 2.0x P.V) increased in parallel to the increase in P.V. the respective values for acidity were 0.98, 1.24 and 1.52 % oleic acid. On the contrary, TAC of oil was decreased with the increase in the storage time. The TAC was 2.22 for virgin olive oil and decreased to 2.15, 2.05 for olive oil preparations with 1.5x P.V and 2.0x P.V, respectively. It is worth noting that (x) refers to the maximum P.V recommended by the Jordanian standard which equals 20meqO_2/kg.

2. Body Weight and Liver Weight

Table 4 shows the initial and final body weights, body weight change and liver weights of rats fed 4% and 8% of either virgin olive oil or stored olive oil preparations (1.5x P.V and 2.0x P.V) for five weeks. Initial body weight were essentially similar ($P>0.05$) in all rats of the experimental groups. All rat groups exhibited similar ($P>0.05$) final body weight and body weight change. Similar results were detected when comparing the effect of virgin olive oil and its preparations on these variables regardless the oil percent (Table 6), and regardless of oil P.V (Table 7). Little change was observed in relative liver weight in all experimental groups (Table 4, 6 and 7). Rats fed diet with 4% stored olive oil preparation with 30 meqO_2/kg exhibited lighter relative liver weights (3.5±0.15) compared to those fed 4% virgin olive oil (4.1±0.09) and 8% stored oil preparation with P.V of 30 meqO_2/kg (4.1±0.03) (Table 4). When comparing

Table 3: Peroxide value, acidity and total antioxidant capacity of the olive oil groups used in the study [1-2].

Oil groups	Peroxide value ($meqO_2$/kg oil)	Acidity (Oleic acid %)	Total Antioxidant capacity (mmol/l)
Virgin olive oil (base line)	10± 0.01[c]	0.98± 0.02[c]	2.22± 0.02[a]
Stored olive oil (1.5x P.V)	30 ± 0.01[b]	1.24± 0.01[b]	2.15± 0.02[b]
Stored olive oil (2.0x P.V)	40± 0.01[a]	1.52 ±0.01[a]	2.05± 0.01[c]

(1) Values represent the mean of triplicates with less than 5% coefficient of variation(mean± SEM)
(2) 1.5x P.V and 2.0x P.V represent 1.5 times and 2.0 times the peroxide value as recommended by the Jordanian standards (P.Vx = 20 $meqO_2$/kg).

Table 4: Initial and final body weights, weight change and liver weight of diabetic rats fed diets contains 4% and 8% of either virgin olive oil or stored oil preparations with peroxide value of 30 and 40 meqO_2/kg for five weeks [(1-4)].

Experimental groups		Initial body weight(g)	Final body weight(g)	Weight change(g)	Liver weight/100g body weight
Virgin olive oil (P.V= 10 meqO_2/kg)	4%	217.6 ± 9.5[a]	190.8 ± 9.8[a]	-26.8 ± 9.8[a]	4.1 ± 0.09[a]
	8%	218.2 ± 8.8[a]	209.7 ±7.4[a]	-8.5 ± 6.6[a]	3.9 ± 0.2[ab]
Stored olive oil (P.V= 30 meqO_2/kg)	4%	214.6 ± 9.5[a]	208.6 ±22.1[a]	-6.0 ± 16.6[a]	3.5 ± 0.15[b]
	8%	216.3 ± 9.1[a]	209.8 ± 22.7[a]	-6.6 ± 19.2[a]	4.1 ± 0.03[a]
Stored olive oil (P.V= 40 meqO_2/kg)	4%	217.5 ± 9.2[a]	197.8 ± 22.8[a]	-19.7 ±21.9[a]	3.6 ± 0.16[ab]
	8%	218.8 ± 4.7[a]	213.4 ±12.6[a]	-5.4 ±9.7[a]	3.9 ± 0.12[ab]

(1) Each value represented as mean ± SEM, n=8 rats.
(2) Values in a column with different superscripts are significantly different ($p<0.05$).
(3) Weight change (g) =final body weight –initial body weight.
(4) P.V: peroxide value.

Table 5: Accumulative food and water intakes and food efficiency ratio of diabetic rats fed 4% and 8% of either virgin olive oil or stored oil preparations with peroxide value of 30 and 40 meqO_2/kg for five weeks [(1-4)].

Experimental groups		Accumulative food intake(g)	Accumulative water intake (l)	Food efficiency ratio
Virgin olive oil (P.V= 10 meqO_2/kg)	4%	1018.8 ± 28[a]	6.1 ± 0.3[a]	-2.7 ± 1.0[a]
	8%	956.2 ± 24.5[a]	5.3 ± 0.2[ac]	-2.5 ± 1.8[a]
Stored olive oil (P.V= 30 meqO_2/kg)	4%	990.8 ± 36.3[a]	5.5 ± 0.5[ac]	-0.4 ± 2[a]
	8%	950.2 ± 38[a]	5.2 ± 0.5[ac]	-0.4 ± 2.5[a]
Stored olive oil (P.V= 40 meqO_2/kg)	4%	948.5 ± 47.8[a]	5.2 ± 0.4[ac]	-2.2 ± 2.7[a]
	8%	932.4 ± 30.7[a]	4.9 ± 0.2[bc]	-0.8 ± 1.1[a]

(1) Each value represented as mean ± SEM, n=8 rats.
(2) Values in a column with different superscripts are significantly different ($p<0.05$).
(3) Food efficiency ratio = body weight change/100g food intake
(4) P.V: peroxide value.

Table 6: Body weights, liver weights, accumulative food and water intake and food efficiency ratio of diabetic rats fed virgin olive oil and stored oil preparations with peroxide value of 30 and 40 meqO_2/kg for five weeks (regardless of oil percent)[1-3].

Variables \ Diet groups	Virgin olive oil (P.V= 10 meqO_2/kg)	Stored olive oil (P.V= 30 meqO_2/kg)	Stored olive oil (P.V= 40 meqO_2/kg)
Initial body weight (g)	217.9 ± 6.3[a]	215.5 ± 6.4[a]	218.2 ± 5.0[a]
Final body weight (g)	200.2 ± 6.4[a]	209.2 ± 15.3[a]	205.6 ± 12.7[a]
Body weight change (g)	-17.7 ± 6.2[a]	6.3 ± 13.1[a]	-12.6 ± 11.7[a]
Liver weight/100g final body weight	4.0 ± 0.11[a]	3.8 ± 0.18[a]	3.8 ± 0.11[a]
Accumulative food intake (g)	987.5 ± 19.7[a]	970.5 ± 25.9[a]	940.4 ± 27.5[a]
Accumulative water intake (l)	5.7 ± 0.19[a]	5.3 ± 0.33[a]	5.1 ± 0.2[a]
Food efficiency ratio	-2.6 ± 1.0[a]	-0.4 ± 1.6[a]	-1.5 ± 1.4[a]

(1) Values are given in mean ± SEM, n=16 rats.
(2) Values with different superscripts within the same row differed significantly ($p<0.05$).
(3) P.V: peroxide value.

Table 7: Body weights, liver weights, accumulative food and water intake and food efficiency ratio of diabetic rats fed 4% and 8% of olive oil for five weeks (regardless of oil peroxide values)[(1-2)].

Variables \ Fat percent	4%	8%
Initial body weight (g)	216.6 ± 5.2[a]	217.8 ± 4.3[a]
Final body weight (g)	199.1 ± 8.6[a]	210.9 ± 10.7[a]
Body weight change (g)	-17.5 ± 9.7[a]	-6.8 ± 7.5[a]
Liver weight/100g final body weight	3.7 ± 0.09[b]	4.0 ± 0.12[a]
Accumulative food intake (g)	986.1 ± 21.9[a]	946.2 ± 17.5[a]
Accumulative water intake (l)	5.6 ± 0.2[a]	5.2 ± 0.2[a]
Food efficiency ratio	-1.7 ± 1.2[a]	-1.2 ± 1.1[a]

(1) Values are given in mean ± SEM, n= 24 rats.
(2) Values with different superscripts within the same row differed significantly ($p<0.05$).

experimental groups regardless of oil P.V (Table 7), rats fed diets with 4% olive oil showed smaller relative liver weights (3.7±0.09) compared to those fed 8% olive oil (4.0±0.12).

Essentially similar results regarding body weight change were observed from weekly data records (Appendix A1), whether within or between the three rat groups, (the virgin olive oil groups and the groups of stored oil preparations with 1.5x P.V and 2.x P.V) for each of the two oil percents (4% and 8%).

It may be noted from these records that body weight change dropped during the first week in all experimental groups, with little or no effect of oil percent or it's P.V.

3. Accumulative Food Intake and Water Intake and Food Efficiency Ratio.

Table 5 shows accumulative food and water intake and food efficiency ratio of diabetic rats fed 4 % and 8% of either virgin olive oil or stored olive oil preparations (1.5x P.V and 2.0x P.V) for five weeks. Accumulative food intake, water intake and food efficiency ratio of all experimental groups did not significantly differ (P>0.05). The same results were obtained when comparing experimental groups regardless of oil percent (Table 6) and oil P.V (Table 7).

Appendices A2,A3 and A4 show, respectively, weekly food intake, water intake and food efficiency ratio of diabetic rats fed 4% and 8% of either virgin olive oil or stored olive oil preparations (1.5x P.V and 2.0x P.V) for five weeks. It may be indicated from these records that these variables did not show significant (P>0.05) changes either intra or inter the six different experimental groups. It may be also indicated that food efficiency ratio did follow the same pattern of that followed by body weight change (Appendix A1) and accumulative food intake (Appendix A2).

4. Serum levels of Glucose, Lipids, and Lipoprotein Cholesterol and Total Antioxidant Capacity.

Table 8 shows serum levels of glucose and lipids profiles and total antioxidant capacity of diabetic rats fed 4% and 8% of either virgin olive oil or stored olive oil preparations (1.5x P.V and 2.0x P.V) for five weeks. No significant differences (P>0.05) were noted in serum levels of glucose, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C) and triglycerides (TG) between the six different experimental groups. Similar results were obtained when comparing the aforementioned variables in all experimental groups regardless oil percent (Table 10) or oil P.V (Table 11).

Regarding TAC, rats fed the 8% stored olive oil preparation (2.0x P.V) showed significantly (P<0.05) higher values (1.18±0.02mmol/l) than those fed 4% of stored oil preparation (1.5x P.V) and 4% virgin olive oil (1.07± 0.03; 1.05±0.03mmol/l respectively). However, TAC level of rats fed 4% virgin olive oil exhibited significantly (P<0.05) lower (1.05±0.03mmol/l) than those fed 8% of stored olive oil preparation with 1.5x P.V. The other experimental groups did not show significant (P>0.05) changes with respect to TAC. When comparing experimental groups regardless of oil percent, TAC did not show significant changes between the different groups (Table 10). However, rats fed 8% olive oil had higher TAC values (1.15±0.02 mmol/l) than those fed 4% olive oil (1.09±0.02 mmol/l) regardless of oil P.V (Table 11).

Table 9 shows indices of serum lipids and lipoprotein- cholesterol of rats fed 4% and 8% of either virgin olive oil or stored olive oil preparations (1.5x P.V and 2.0x P.V) for five weeks. There were no significant differences (P>0.05) in LDL-C/HDL-C and LDL-C/ TG ratios as well as in atherogenic index between all different experimental groups, Insignificant differences (P>0.05) in these variables were also obtained between

Table 8: Serum levels of glucose, lipids and total antioxidant capacity of diabetic rats fed 4% and 8% of either virgin olive oil or stored oil preparations with peroxide values of 30 and 40 meqO_2/kg for five weeks[(1-3)].

Experimental groups		Glucose (mg/dl)	Total cholesterol (mg/dl)	Low-density lipoprotein cholesterol (mg/dl)	High-density lipoprotein cholesterol (mg/dl)	Triglyceride (mg/dl)	Total antioxidant capacity (mmol/l)
Virgin olive oil (P.V= 10 meqO_2/kg)	4%	425.7 ± 52.7[a]	110.9 ± 10.6[a]	24.7 ± 8.4[a]	75.9 ± 3.8[a]	123.1 ± 21.2[a]	1.05 ± 0.03[bd]
	8%	451.7 ± 47.6[a]	119.6 ± 9.1[a]	27.6 ± 5.2[a]	76.3 ± 4.3[a]	124.1 ± 32.3[a]	1.1 ± 0.04[acb]
Stored olive oil (P.V= 30 meqO_2/kg)	4%	449.2 ± 48.7[a]	99.9 ± 7.5[a]	26.5 ± 3.7[a]	72.0 ± 1.1[a]	102.3 ± 21.8[a]	1.07 ± 0.03[bcd]
	8%	462.1 ± 54.8[a]	122.1 ± 16.8[a]	30.1 ± 6.2[a]	78.6 ± 9.7[a]	124.1 ± 20.6[a]	1.17 ± 0.03[ac]
Stored olive oil (P.V= 40 meqO_2/kg)	4%	433.3 ± 63.2[a]	122.9 ± 20.5[a]	34.1 ± 9.1[a]	70.4 ± 10.6[a]	103.7 ± 41.7[a]	1.13 ± 0.05[acd]
	8%	443.6 ± 45.8[a]	118.9 ± 10.3[a]	34.1 ± 7.9[a]	73.2 ± 6.2[a]	103.3 ± 13.4[a]	1.18 ± 0.02[a]

(1) Each value represented as mean ± SEM, n=8 rats.
(2) Values in a column with different superscripts are significantly different ($p<0.05$).
(3) P.V: Peroxide value.

Table 9: Indices of serum lipids and lipoprotein –cholesterol of diabetic rats fed 4% and 8% of either virgin olive oil or stored oil preparations with peroxide values of 30 and 40 meqO_2/kg for five weeks [1-5].

Experimental groups		LDL-C/HDL-C	LDL-C/TG	Atherogenic index
Virgin olive oil (P.V= 10 meqO_2/kg)	4%	0.31 ± 0.09 [a]	0.23 ± 0.09 [a]	0.45 ± 0.1 [a]
	8%	0.36± 0.07[a]	0.39 ± 0.16 [a]	0.58 ± 0.13 [a]
Stored olive oil (P.V= 30 meqO_2/kg)	4%	0.37 ± 0.05 [a]	0.34 ± 0.09 [a]	0.38 ± 0.09 [a]
	8%	0.37 ± 0.05 [a]	0.26 ± 0.05 [a]	0.58 ± 0.16 [a]
Stored olive oil (P.V= 40 meqO_2/kg)	4%	0.76 ±0.31 [a]	0.39 ± 0.14 [a]	1.18 ± 0.61 [a]
	8%	0.54 ± 0.17 [a]	0.32 ± 0.05 [a]	0.71 ± 0.24 [a]

(1) Each value represented as mean ± SEM, n=8 rats.
(2) Values in a column with different superscripts are significantly different ($p<0.05$).
(3) Atherogenic index = (TC-HDL-C) / HDL-C (Martin-Carron *et al*., 1999).
(4) P.V: Peroxide value.
(5) LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides.

Table 10: Serum levels of glucose, lipids, lipoproteins and their indices and total antioxidant capacity of diabetic rats fed virgin olive oil and stored oil preparations with peroxide values of 30 and 40 $meqO_2/kg$ for five weeks (regardless of oil percent) [(1-5)].

Diet groups / Variables	Virgin olive oil (P.V= 10 $meqO_2/kg$)	Stored olive oil (P.V= 30 $meqO_2/kg$)	Stored olive oil (P.V= 40 $meqO_2/kg$)
Glucose	438.8 ± 34.6 [a]	455.7 ± 35.5 [a]	438.5 ± 32.4 [a]
TC	115.3 ± 6.8 [a]	111.0 ± 9.4 [a]	120.9 ± 11.1 [a]
LDL-C	26.1 ± 4.8 [a]	28.3 ± 3.5 [a]	34.1 ± 5.8 [a]
HDL-C	76.2 ± 2.8 [a]	75.3 ± 4.8 [a]	71.8 ± 5.9 [a]
TG	123.6 ± 18.7 [a]	113.2 ± 14.8 [a]	103.5 ± 21.2 [a]
TAC	1.08 ± 0.03 [a]	1.13 ± 0.03 [a]	1.16 ± 0.03 [a]
LDL-C/ HDL-C	0.34 ± 0.06 [a]	0.37 ± 0.03 [a]	0.65 ± 0.17 [a]
LDL-C / TG	0.31 ± 0.09 [a]	0.29 ± 0.05 [a]	0.39 ± 0.08 [a]
Atherogenic index	0.52 ± 0.08 [a]	0.48 ± 0.08 [a]	0.94 ± 0.32 [a]

(1) Values are given in mean ± SEM, n=16 rats.
(2) Values with different superscripts within the same row are significantly different ($p<0.05$).
(3) P.V: peroxide value.
(4) LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; TAC: total antioxidant capacity; TC: total cholesterol.
(5) Atherogenic index = (TC-HDL-C)/HDL-C (Martin-Carron *et al*., 1999).

Table 11 : Serum levels of glucose, lipids, lipoproteins and their indices and total antioxidant capacity of diabetic rats fed 4% and 8% of olive oil for five weeks (regardless of oil peroxide values) [(1-5)].

Variables \ Fat percent	4%	8%
Glucose	436.1 ± 29.3 [a]	452.5 ± 25.8[a]
TC	111.2 ± 7.9 [a]	120.2 ± 6.9 [a]
LDL-C	28.5 ± 4.2 [a]	30.6 ± 3.7[a]
HDL-C	72.8 ± 3.6 [a]	76 ± 3.9 [a]
TG	109.7 ± 16.6 [a]	117.2 ± 13.1 [a]
TAC	1.09 ± 0.02 [b]	1.15 ± 0.02[a]
LDL-C/ HDL-C	0.48 ± 0.11 [a]	0.42 ± 0.06[a]
LDL-C / TG	0.35± 0.06[a]	0.32 ± 0.06[a]
Atherogenic index	0.67± 0.21[a]	0.63 ± 0.1[a]

(1) Values are given in mean ± SEM, n= 24 rats.
(2) Values with different superscripts within the same row are significantly different ($p<0.05$).
(3) P.V: peroxide value.
(4) LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; TAC: total antioxidant capacity; TC: total cholesterol.
(5) Atherogenic index = (TC-HDL-C)/HDL-C (Martin-Carron *et al*., 1999).

rat groups fed olive oil with different P.V regardless of oil percent (Table10) and those fed 4% and 8% olive oil regardless of oil P.V (Table 11).

6. Pearson's correlation coefficients for some variables

Table 12 shows person's correlation coefficients for selected variables of diabetic rats fed 4% and 8% of either virgin olive oil or stored olive oil preparations (30 P.V and 40 P.V) for five weeks. A significant positive correlation was obtained between serum levels of glucose and each of accumulative food intake($r = 0.33$, $P<0.05$), accumulative water intakes ($r = 0.43$, $P<0.01$), and relative liver weight ($r = 0.49$, $P<0.01$). Similarly, significant positive correlations were obtained between the serum levels of total cholesterol and the following variables: LDL-C ($r = 0.64$, $P<0.01$), triglycerides ($r = 0.55$, $P<0.01$), and TAC ($r = 0.39$, $P<0.01$). Significant positive correlations were also found between the LDL-C and triglycerides ($r = 0.31$, $P<0.05$) and between TAC and triglycerides ($r = 0.35$, $P<0.05$), as well as between the relative liver weight and HDL-C ($r = 0.38$, $P<0.01$). Furthermore, a significant positive correlation was noticed between the accumulative food intake and accumulative water intake ($r = 0.81$, $P<0.01$) and between weight change and food efficiency ratio ($r = 0.93$, $P<0.01$).

On the other hand, significant negative correlations were observed between the following variables: between serum glucose and food efficiency ratio ($r = -0.42$, $P<0.01$) and weight change ($r = -0.39$, $P<0.01$); between TC and food efficiency ratio ($r = -0.34$, $P<0.05$) and weight change ($r = -0.36$, $P<0.05$); between LDL-C and each of accumulative food intake ($r = -0.37$, $P<0.01$) and food efficiency ratio ($r = -0.42$, $P<0.01$) and weight change ($r = -0.42$, $P<0.01$); between TAC and accumulative food intake ($r = -0.35$, $P>0.05$) and accumulative water intake ($r = -0.42$, $P>0.01$); between relative liver weight and weight change ($r = -0.36$, $P>0.05$) and food efficiency ratio ($r = -0.33$, $P>0.05$);

Table 12: Pearson's correlation coefficients for selected variables in diabetic rats fed diets containing 4% and 8% of either virgin olive oil or stored oil preparation with peroxide values of 30 and 40 meqO_2/kg for five weeks [1-3].

Variables	Glucose	TC	LDL-C	HDL-C	TG	TAC	RLW	AFI	AWI	FER	WC
Glucose	----										
TC	**r =0.04**	----									
LDL-C	**r =-0.05**	**r = 0.64****	----								
HDL-C	**r =0.24**	**r = 0.28**	**r =-0.1**	----							
TG	**r = 0.03**	**r = 0.55****	**r=0.31***	**r=-0.02**	----						
TAC	**r = -0.11**	**r = 0.39****	**r=0.25**	**r=0.18**	**r=0.35***	----					
RLW	**r = 0.49****	**r =-0.16**	**r =-0.07**	**r =0.38****	**r =0.14**	**r =0.18**	----				
AFI	**r =0.33***	**r =-0.14**	**r =-0.37****	**r =0.22**	**r =-0.02**	**r =-0.35***	**r =0.2**	----			
AWI	**r =0.43****	**r =-0.05**	**r =-0.14**	**r =0.07**	**r =-0.01**	**r =-0.42****	**r =0.03**	**r =0.81****	----		
FER	**r =-0.42****	**r =-0.34***	**r =-0.42****	**r =0.02**	**r =0.02**	**r =0.12**	**r =-0.33***	**r =-0.01**	**r =-0.26**	----	
WC	**r =-0.39****	**r =-0.36***	**r =-0.42****	**r =0.04**	**r =-0.01**	**r =0.19**	**r =-0.36***	**r =-0.04**	**r =-0.3***	**r=0.93 ****	----

(1) Abbreviations: Total Cholesterol(TC), Low Density Lipoprotein-Cholesterol(LDL-C), High Density Lipoprotein- Cholesterol(HDL-C), Triglycerides(TG),Total Antioxidant Capacity(TAC),Relative Liver Weight (RLW),Accumulative Food Intake(AFI), Accumulative Water Intake(AWI),Food Efficiency Ratio(FER) and Weight Change(WC).
(2) *, ** Represent significant difference at the 0.05 and 0.01 probability levels, respectively.
(3) N=48.

and between accumulative water intake and weight change ($r = -0.30$, $P > 0.05$). None of the other correlations obtained were significant ($P > 0.05$).

Discussion

Studies conducted to investigate the effect of olive oil either virgin olive oil or stored olive oil on the serum glucose, lipids and lipoprotein levels in diabetes mellitus are very limited. This is perhaps the first study examining the effect of virgin olive oil and stored oil preparations on serum TAC in diabetes. Such limitations make the discussion of the result difficult. However; it may be beneficial to open a new avenue of thinking for future research and studies in this field. It is important to emphasize that the obtained results are related to the conditions followed by the present study, and any change in these conditions, especially the lipid material and its levels may affect the results.

Schaal test was used to evaluate the oxidation of olive oil during accelerated storage conditions (velasco and Dobarganes, 2002). The conditions followed by the test usually simulate the real storage conditions in the market or at home. This test was originally designed to evaluate the stability of shortening in baked products. It is a simple shelf-storage test with an evaluated temperature to speed up the process of fat oxidation. A common test temperature is 62.8C^{o}. Periodic odor and flavor evaluations were commonly used. Chemical analysis of Peroxide value may be used to determine rancidity development in the samples.

In the present study Table (3), the peroxide value (P.V) and acidity were increased linearly during the storage time. It needed 40 days to changing P.V from 10 to 40 meqO_2/ kg oil. The length of this period may be attributed to the high oxidative stability of virgin olive oil (Cinquanta, *et al.,* 2001). This period may differ from oil to oil; depending upon the composition and antioxidant contents (Cinquanta, *et al*., 2001). Storage conditions followed by the present study were responsible for decreased antioxidant content of the tested olive oil, as shown in Table (3). The total antioxidant

capacity (TAC) was decreased from 2.22 to 2.05 mmol/L. Bendini, *et al*. (2006) found that exposing virgin olive oil to accelerated storage test at 60C^{o} for six weeks resulted in a decrease in the antioxidant activity of the oil from 1.17 to 0.93 mmol/L. Pellegrini *et* al., (2003) reported that soy bean oil has higher antioxidant capacity than extra virgin olive oil (2.2 and 1.79 mmol/L, respectively), which may be due to its high tocopherol content.

Animals receiving streptozotocin (STZ) exhibit metabolic and clinical manifestations reminiscent of human diabetes (Szkudelski and Skudelska, 2002). The diabetes induced by STZ is associated with polydepsia, polyuria, polyphagia, and weight loss (Yang *et al*., 2008). There were little or no reported observations about the effects of olive oil either virgin or stored on the body weight, liver weight, food and water intake and food efficiency ratio in diabetic rats.

As shown in (Table4), there was no significant difference ($P>0.05$), as expected, in the initial body weight between all experimental groups. At the end of experiment, the difference in final body weight and weight change was not significant among all rat groups. In this study, all experimental groups showed a decrease in body weight. However, significant negative correlation was observed between weight change and serum glucose (Table12)

Body weight loss was one of the characteristics of STZ- diabetic rats that have been inducted by Zhong, *et al*. (2001), Namkoong, *et al*. (2005), and Altibi, (2010). They reported that STZ injected rats had significantly ($P<0.05$) lower mean body weight compared with the control rats. STZ is a nitrosurea-glucose derivative that induces insulin deficiency via selectively destroying pancreatic β cells. STZ treated animals are expected to exhibit physical wasting, hyperglycemia, glucoseuria, and hyperlipidemia reminiscent of human diabetes (Rabinowtiz and Craig, 1989; Banner-weir, *et al.,* 1981).

The classical hypoinsulinimia and concomitant hyperglucagonemia in diabetes account, indeed, for higher catabolic rates of body energy stores including fat and muscular protein as well as glycogen (Lewis, *et al.,* 2002). It is apparent that diabetes- induced catabolic state hampered any gain in weight despite having significantly higher food intake.

In the present study, liver weight was fairly affected by the different oil treatments (Table 4). Rats fed 4% virgin olive oil and 8% of stored olive oil (1.5x P.V) had significantly ($P<0.05$) higher relative liver weight than those fed 4% of stored oil (1.5x P.V). Liver plays an important role in glucose and lipid metabolism (Eidi and Eidi, 2009). The liver weight of diabetic rats was higher than those of the non diabetic rats (Cho, *et al.,* 2002; Giron, *et al.,* 1999). During diabetes a profound alteration in the concentration and composition of lipids occurs (Gupta, *et al.,* 1999; Giron, *et al*., 1999).

Food intake increased with STZ treatment. This fact has been indicated by Namkoog, *et al*. (2005), Qasem, (2004), and Ibrahim, (2007). As shown in Table (5), there was no significant ($P>0.05$) difference in the accumulative food intake between all rat groups. However, there was a positive correlation between accumulative food intake and serum glucose. Haddad, (2005) found that increasing oil percent in corn and olive oil diet groups decreased the rats food intake in normal rats. This could mean that increase oil percent in diet has an effect on the appetites of rats.

Food efficiency ratio, which expressed as weight change (g)/ 100g total food intake, reflectes differences in weight change related to food consumption. In the present study, there were no significant differences ($P>0.05$) in food efficiency ratio among all experimental groups (Table5). All rats, however, showed a decrease in food efficiency ratio. These results are in accordance with results previously reported after STZ treatment of diabetic rats (Altibi, 2010). This may be due to disturbance in the food

intake induced by metabolic aberrations and a decrease in body weight. In this study, food efficiency ratio negatively correlated well with food intake and positively related to weight change (Table 12).

Although all rats, in this study, exhibited marked polyuria, water intake was not significantly affected by the different oil treatments (Table 5). Significant positive correlations were obtained between water intake and food intake and serum glucose (Table 12). Water intake also correlated negatively with weight change. Similar results were reported by Qasem, (2004).

In this study, STZ-diabetic rats were used to examine the influence of varying proportions of virgin and stored olive oil (exposed to air and light-heat) in the diet on the levels of serum total antioxidant capacity, glucose, lipids, and lipoprotein cholesterol. Diabetic rats exhibited an increase in the levels of blood glucose (Namkoong, *et al*., 2005; Szkudelsdi and Szkudelska, 2002), cholesterol, triglycerides, LDL-C (Alhazza, 2007; Cho, *et al.,* 2002), lipid peroxidation (Jemai, *et al.,*2009) and decrease in the level of HDL-C (Alhazza, 2007; Cho, *et al.,* 2002) and depletion in the antioxidant enzymes activities (Jemai, *et al.,*2009).

Diet is the cornerstone managment for patients with type II diabetes mellitus. The main aim of the diet is to normalize the derangements in intermediary metabolism in patients with diabetes mellitus such as hyperglycemia and hyperlipidemia. Another objective of diet therapy is to prevent long-term complications of diabetes mellitus.

Oxidative stress generated by hyperglycemia or by hyperlipidemia and decline of antioxidative defense mechanisms is regarded as an important mediator for diabetic complications (Martin-Gallan, *et al.,* 2003). Hyperglycemia and hyperlipidemia lead to multiple metabolic defects leading to increased production of reactive oxygen species (Chiasson, 2006). Reactive oxygen species are known to be responsible for oxidative

damage of DNA, nucleotides, proteins, lipids, carbohydrates, and cell membrane structure (Piwowar, *et al*., 2007).

Olive oil is effective against oxidative stress associated disease. Besides it's richness in monounsaturated fatty acids, the oleic acid, olive oil contains minor component with antioxidant properties (Fitó, *et al.,* 2007). The oleic acids is a major component of olive oil has antioxidant effect (Reaven, *et al.,*1993) and it is responsible for decreasing LDL-C, cholesterol, triglycerides levels of hypercholesterolemic patients (Sirtori, *et al.,*1992). The administration of diets enriched in oils rich in unsaturated fatty acids was shown to reduce the tissues to produce lipid peroxides (LÁbbe, *et al.,* 1991).

Phenolic compounds in olive oil have shown antioxidant properties that prevent lipoperoxidation, and induce favorable changes of lipid profile (López-Miranda, *et al.,* 2010). The phenolic compounds in virgin olive oil can reduce cardiovascular risk factor levels, the EUROLIVE study provided clear evidence that virgin olive oil is more than just MUFA. In that study, olive oils with different phenolic content were given, and all of them reduced serum triglycerides and increased HDL-C and reduced oxidized glutathione ratio. However, the increased HDL-C and reduced oxidative damage relate to the lipids was related to the phenolic content of virgin olive oil in a dose-dependent manner (Covas, *et al.,* 2006a).

Exposure of oil to heat and air-light may alter its composition especially fatty acid and antioxidant contents, a matter that may affect olive oil health benefits (Fito, *et al.,* 2005; Naz, *et al.,* 2005). Generally olive oil phenolic is subject to degradation upon heating (Cicerale, 2009).

In the present study, there were no significant differences in serum glucose and lipid profiles among all experimental groups. The same result was detected when

comparing between P.Vs and also when comparing between oil percents. This may mean that virgin olive oil and stored olive oil could contain a heat-resistant compound that has beneficial effects on serum glucose and lipid profiles, or that the metabolic defects induced by STZ diabetes are far deeper than that to be affected by the present oil treatments. Similarly results were reported by Ahmad and Khatib (1990) who found that there is no effect of dietary fat (corn oil, olive oil cod-liver oil, sheep tallow, and lard) on the plasma glucose and lipid profiles in the STZ diabetic rats. Alhazza.(2007) studied the effect of olive oil on normal and diabetic rats. The result showed that glucose, TG, TC, LDL-C, and hydroperoxide levels in diabetic olive oil group were significantly ($P<0.05$) lower than diabetic control group, and the HDL-C level was higher in olive oil group. The same results were obtained in the case of normal (non-diabetic) groups.

Whereas the total antioxidant capacity (TAC) of stored olive oil decreased, serum TAC of rats given this oil increased. As shown in Table (8), serum TAC of rats fed 8% stored (2.0x P.V) olive oil was significantly ($P<0.05$) higher than those fed 4% virgin olive oil and 4% stored olive oil (1.5x P.V) groups. Values of serum TAC of rats fed 4% virgin olive oil were significantly ($P<0.05$) lower than those fed 8% of both stored olive oil (1.5x P.V and 2.0x P.V).

These results may be explained in view of increased oxidative stress due to feeding stored oil, that leads to increased free radical production, and this in turn needs more antioxidants to prevent the increase in the lipid peroxidation in diabetic rats. On the other hand, the effect of olive oil on serum lipid profile was little or even unclear in this study, and so that on serum TAC. This may be due to uncontrolled hyperglycemia in diabetic rats. Several studies show the decline of serum total antioxidant capacity in diabetic patients (Nobar, *et* al., 1999; Seghrouchni, *et* al., 2002). Al-Behari, (2010) found that giving 20g of black cumin seeds either roasted or raw to diabetic patients

(Type2) did not affect serum total antioxidant capacity, although black cumin seeds are rich in antioxidants.

A significant increase in lipid peroxidation in diabetic rats suggests an increased generation of free radicals by hyperglycemia related to glucose auto-oxidation (Woeff and Dean, 1987). After administration of olive oil, the levels of lipid peroxides declined significantly in both normal and diabetic rats (Aguilera, *et al.,* 2003). Long-term regular intake of virgin olive oil consumption protected the endogenous antioxidant system (Valls, *et al.,* 2010).

In the present study, changing P.V of the olive oil should be accompanied with the loss of antioxidant contents of the oil. However, serum glucose, lipid and lipoprotein levels in diabetic rats were not affected by changing P.V. This means that some of the components of olive oil were not changed by the accelerated storage conditions followed by the present study. Allouche, *et al*. (2007) exposed the extra virgin olive oil to thermal treatment (180C^{o} for 36h), and they found that tocopherol and polyphenols were the most affected by the thermal treatment. On the other hand, oleic acid, sterols, squalene, and triterperic alcohols (erythrodiol and uvaol) and acids (oleanolic and maslinic) were quite constant, exhibiting a high stability against oxidation. Pellegrini *et al*. (2001) found that increasing polyphenol content in oils protects α-tocopherol from being depleted during heating.

Administration of virgin olive oil to normal rats led to decreased levels of plasma cholesterol, triglycerides, phospholipids, total lipids, and DNA double-strand breaks, and a very good inverse correlation between DNA damage and total antioxidant capacity were found (Quiles, *et al.*, 2004). Jemai *et al*., (2009) found that administration of hydoxytyrosol and oleuropein to diabetic rats led to reduced serum glucose and cholesterol levels and restored the antioxidant perturbations. Hamden, *et al* (2009)

found that the olive mill waste polyphenols and hydroxytrosol were efficient in inhibiting hyperglycemia and oxidative stress induced by diabetes and they suggested that administration of hydroxytyrosol may be helpful in the prevention of diabetic complication associated with oxidative stress

Nakbi, *et al*. (2010) studied the effect of extra virgin olive oil on the antioxidant status and fatty acid profile in the erythrocyte of 2,4-dichlorophenoxyacetic acid (2,4-D) exposed rats. The 2, 4-D produces oxidative stress and /or depletes antioxidants both *in vitro* and *in vivo*. Extra virgin olive oil protected erythrocyte membranes against oxidative damage by preventing excessive lipid peroxidation by increasing the MUFA composition and maintaining antioxidant enzymes at normal concentration.

Castaňer, *et al.* (2011) divided 200 healthy men randomly to three groups, each groups took 25 ml/day of three olive oil with high, medium, and low phenolic content for three weeks. Plasma oxidatized LDL autoantibodies was inversely associated with oxidized LDL, with increased both phenolic content, and oxidatized LDL autoantibodies generation (oxidatized LDL is a highly immunogenic particle that play an important role in the development of atherosclerosis).

Dietary antioxidants have been hypothesized to have a protective effect against the development of diabetes by inhibiting peroxidation chain reactions (Maritim, *et al.,* 2003). Yang, *et al.* (2006) studied the effect of antioxidant like compounds (Lipoic acid and N-acetylcysteine) on the serum lipid profile and lipoprotein lipase activity in rats fed high fat diets for 4 weeks. They found that the high fat diet led to increased in the lipid peroxidation, serum TC, TG, LDL-C and decreased HDL-C. They also found that administration of antioxidants can improve the antioxidant capacity and the activity of lipoprotein lipase, and reduce blood lipids. In another study showed that the intakes of total vitamin E, α-tocopherol, γ- tocopherol ,β-tocotrienol, and β-cryptoxanthin were

associated with reduced risk of type 2 diabetes, and there was no association between vitamin C and type 2 diabetic risks (Montonen, *et al*., 2004).

There were no differences in LDL-C/HDL-C ratio among all treatment groups (Table 9). The LDL-C/HDL-C ratios in all experimental groups were less than 1; this means that serum HDL-C levels were higher than LDL-C concentrations. This indicates that olive oil in this study either virgin or stored has antiatherogenic effect on the diabetic rats. HDL-C can be protective by reversing cholesterol transport, inhibiting the oxidation of LDL-C and by neutralizing the atherogenic effects of oxidized LDL-C. HDL-C helps to scavenge cholesterol from extra hepatic tissues (Glew, 2006). LDL-C/TG ratios were also unaffected by the present study treatments in all experimental groups. LDL-C/ TG ratios were less than 1; this means that TG value was higher than LDL-C value. This may be due to increased lipolysis in adipose tissue in the absence of insulin, and decreased in lipoprotein lipase activity (Maghrani, *et al.,*2004; Eidi and Eidi, 2009).

The Atherogenic index, defined as the ratio of TC-HDL-C/ HDL-C, is believed to be an important risk factor of atherosclerosis (Takasaki, 2005; Yang, *et al*., 2006). Atherogenic index of diabetic rats was significantly higher than normal rats. Cho, *et al.* (2002) found that the atherogenic index in non diabetic rats was 1.95, and in diabetic rats were 8.44. Abdoh, (2006) found normal rats have 0.41 atherogenic index, and Yang *et al*. (2006) reported 0.70 for normal rats. In the present study, the atherogenic index is quite close to normal level for most rat groups (Table 9). Atherogenic index of rats fed stored olive oil (2.0x P.V) tended to be higher than those fed virgin olive oil and stored olive oil (1.5x P.V). This means that administration of olive oil either virgin or stored has appositive effect on the atherogenic index. Yang, *et al*., (2006) found a negative

correlation between atheroginic index and serum total antioxidant status and a positive one between total antioxidant status and lipoprotein lipase.

Conclusion and Recommendation

- Storing virgin olive oil led to a marked increase in peroxide value and acidity with a substantial parallel decrease in its total antioxidant capacity.
- Streptozotocin-induced diabetes induced marked metabolic derangements that led to hyperglycemia; body weight loss and higher food and water intakes.
- Feeding virgin olive oil and stored preparations produced similar effect on body weight, liver weight, food efficiency ratio, and food and water intakes.
- Feeding virgin olive oil or its stored preparations (4% or 8%) did not significantly affect serum levels of glucose, TC, HDL-C, LDL-C, TG and ratios of LDL-C/ HDL-C and LDL-C/ TG and Atherogenic index.
- Regardless of both oil percent and oil peroxide value, none of the serum variables or lipid fractions was influenced by olive oil feeding.
- Feeding virgin olive oil or its stored preparations enhanced the total antioxidant capacity of the diabetic rats.
- Serum TAC correlated positively with TC and TG and negatively with food intake and water intake. Serum glucose related positively to food intake and water intake and negatively to weight change and food efficiency ratio.
- Serum TC and LDL-C correlated negatively with weight change and food efficiency ratio. Food intake related well to weight change and food efficiency ratio.
- It is suggested that further studies may be carried out to examine the effect of cooking and frying, with olive oil, on the diabetes, metabolic and clinical variables.

- Human studies are needed to investigate the effect of virgin olive oil and stored olive oil on the bio and clinical markers in normal and diabetic people.

References

Abdoh, M.J.(2006), **Effect of Birhi Variety of Date Palm Fruits, *Phoenix Dactylifera* L. at the Khalal and Tamr Stages on Blood Lipid Levels in Rats Fed Cholesterol Supplemented Diets**. Unpublished master thesis, University of Jordan, Amman, Jordan.

Ahmad , M.N. and khatib, F.A. (1990), Effect of Dietary Saturated, Monounsaturated, and Polyunsaturated Fats on Plasma Lipids and Lipoproteins in Diabetic Rats. **Ecology of Food and nutrition,** 24, 141-148.

Agilera, C.M., Mesa, M.D., Ramirez-Tortosa, M.C.,Quiles, J.L., Gil, A.(2003), Virgin Olive and Fish Oils enhance the Hepatic Defense System in Atherosclerotic Rabbits. **Clinical Nutrition,** 22(4), 379-384.

Ajlouni, K., Khader, Y.S., Batieha, A., Ajlouni, H. and El-Khateeb, M. (2008), An Increase in Prevalence of Diabetes Mellitus in Jordan Over 10 Years. **Journal of Diabetes and its Complications**, 22,317-324.

Ajlouni, K., Qusous, Y., Khawaldeh, A.K., Jaddou, H., Batieha, A., Ammari, F., Zaheri, M. and Mashal, A.(1999), Incidence of Insulin-Dependent Diabetes Mellitus in Jordanian Children Age 0-14 During 1992-1996. **Acta Paediatrica Supplement**, 88(427), 11-13.

Al-Behari, S.H.(2010),**Effect of Black Cumin Seeds(*Nigella Sativa L.*) on Blood Glucose, Haemoglobin A1C and Antioxidant Capacity in a Group of Jordanians with Type 2 Diabetes Mellitus**, Unpublished master thesis, University of Jordan, Amman, Jordan.

Alhazza, I.M. (2007), Antioxidant and Hypolipidemic Effects of Olive Oil in Normal and Diabetic Male Rats. **Saudi Journal of Biological Sciences**, 14(1), 69-74.

Allouche, Y., Jiménez, A., Gaforio, J.J., Uceda, M. and Beltrán, G. (2007), How Heating Affects Extra Virgin Olive Oil Quality Indexes and Chemical Composition, **Agricultural and Food Chemistry**, 55, 9646-9654.

Alsaif, M.A. and Duwaihy, M.S.(2004), Influence of Dietary Fat Quantity and Composition on Glucose Tolerance and Insulin Sensitivity in Rats. **Nutrition Research**, 24, 417-425.

Altibi, A.M.(2010), **Effect of Dehulled and Cooked Lentils(*Lens Culinaris,L.)* on Serum Glucose and Lipoprotein Levels in Streptozotocin-Induced Diabetic Rats**, unpublished master thesis, University of Jordan, Amman, Jordan.

American Diabetes Association. (2008), Nutrition Recommendations and Interventions for Diabetes .**Diabetes Care**, 31, S61-S78.

American Diabetes Association. (2006), Diagnosis and Classification of Diabetes Mellitus. **Diabetes Care**, 29, S43-S48.

American Diabetes Association. (2002), Evidence Based Nutrition Principles and Recommendations for the Treatment and Prevention of Diabetes and Related Complications, **Diabetes Care,** 25, S50-S60.

Anderson, J.W. (2006), Diabetes Mellitus: Medical Nutrition Therapy. In: Shils, M.E., Shike, M., Ross, A.C., Caballero, B. and Cousins, R., **Modern Nutrition in Health and Disease**, 10^{th}ed. (PP.1043-1066) Philadelphia, USA: Lippincott Williams and Wilkins.

Asian-Pacific Type2 Diabetes Policy Group (2002), **Type 2 Diabetes Practical Target and Treatments**, (3^{rd} ed), Australia.

Association of Official Analytical Chemists. (1995).**Official Methods of Analysis of the Association of Official Analytical Chemists**. AOAC, Virginia, USA.

Baynes, J.W. and Thorpe,S.R. (1999), Role of Oxidative Stress in Diabetic Complications: a new Perspective on an old Paradigm. **Diabetes,** 48, 1-9.

Bendini, A., Cerretani, L., Vecchi, S., Carrasco-Pancordo, A. and Lercker, G.(2006), Protective Effects of Extra Vergin Olive Oil Phenolics on Oxidative Stability in the Presence or Absence of Copper Ions **, Agricultural and Food Chemistry**, 54, 4880-4887.

Boden, G. and Shulman, G.I.(2002), Free Fatty Acids in Obesity and type 2Diabetes: Defining their Role in the Development of Insulin Resistance and beta-cell Dysfunction. **Eur. Journal of Clinical Investigate**, 32(3), 14-23.

Bojestig, M., Arnqvist, H.J., Hermansson, G., Karlberg, B.E. and Loudvigsson, J. (1994), Declining Incidence of Nephropathy in Insulin-Dependent Diabetes Mellitus. **N Engl J Med.** 330, 15-18.

Bondia-Pons, I., Schroder, H., Covas, M.I., Castellote, A.I., Kaikkonen, J., Poulsen, H.E., Gaddi, A.V., Machowetz, A., Kiesewetter, H. and Lopez-Sabater, M.C.(2007), Moderate Consumption of Olive Oil by Healthy European Men Reduces Systolic Blood Pressure in non-Mediterranean Participants. **Journal of Nutrition**, 137(1), 84-87.

Bonner-Weir, S., Trend, D.F., Honey, R.N. and Weir, G.C. (1981). Responses of Neonatal Rat Islet to Streptozotocin. **Diabetes**, 30, 64-69.

Boskou, D. (2000). Olive oil. **World Review of Nutrition and Diabetics**, 87, 56-77

Brownlee, M. (2001), Biochemistry and Molecular Cell Biology of Diabetes Complications. **Journal of Nutrition.** 414, 813-820.

Cabello-Moruno, R., Perona, J.S., Ruiz-Gutierrez, V. (2007), Influence of Minor Components of Olive Oils on the Composition and Size of TRLs, and on macrophage Receptors involved in foam cell Formation. **Biochem. Soc Trans**, 35, 470-471.

Carluccio, M.A., Massaro, M., Scoditti, E., De Caterina, R. (2007), Vasculoprotective Potential of Olive Oil Components. **Molecular Nutrition & Food Research**, 51, 1225-1234.

Castañer, O., Fitó, M,. López-Sabater, M.S., Poulsen, H.E., Nyyssönen, K., Schröder, H., Salonen, J.T. ,Torre-Carbot, K.D., Zunft, H., Torre, R.D., Bäumler, H., Gaddi, A.V., Saez, G.T., Tomás, M. and Covas, M .for the EUROLIVE study group.(2011), The Effect of Olive Oil Phenols on Antibodies Against Oxidized LDL a Randomized Clinical Trial, **Clinical Nutrition**, 30, 490-493.

Cavalcante, A.G. and Bruin, P.F. (2009), Role of Oxidative Stress in COPD: Current Concepts and Perspectives. **Journal Brasileirs de Pneumologia**, 35, 1806-1813.

Centers for Disease Control and Prevention (CDC). (2005), **National Diabetes Fact Sheet, CDC Publication no, 20**, USA.

Centers for Disease Control and Prevention (CDC) (2008), **Diabetes, Disabling Disease to Double by 2050.** USA.

Chiasson, J (2006). Oxidative Stress in the Development of Diabetes and its Complications. In: Bourassa, Martial G; Tardif, Jean- Claude (Eds). **Antioxidants and Cardiovascular Medicine,** 2[ed] edition, USA, Speinger.

Cho, S., Park, J., Park, E., Choi, M., Lee, M., Jeon, S., Jang, M. K., Kim, M. and Park, Y. B.(2002), Alteration of Hepatic Antioxidant Enzyme Activities and Lipid Profile in Streptozotocin-Induced Diabetic Rats By Supplementation of Dandelion Water Extract, **Clinica Chimica Acta**, 317, 109-117.

Cicerale, S., Conlan, X.A., Barnett, N.W., Sinclair, A.J. and Keast, R.S.J.(2009), Influence of Heat on Biological Activity and Concentration of Oleocanthal- a Natural Anti-inflammatory Agent in Virgin Olive Oil. **Agric. Food. Chem,** 57, 1326-1330.

Cinquanta, L., Esti, M. and Matteo, M.D.(2001), Oxidative Stability of Virgin Olive Oil. **JAOCS**, 78, 1197-1202.

Covas, M.I., Nyyssonen, K., Poulsen, H.E., Kaikkonen, J., Zunft, H.J., Kiesewetter, H**. et al (**2006a), The Effect of Polyphenols in Olive Oil on Heart Disease Riske Factors :A Randomized Trial. **Ann Intern Med**, 145, 333-341.

Covas, M-I., Ruiz-Gutierrez, V., de la Torre, R, Kafatos, A., Lamuela-Reventos, R.M., Osada, J., Owen, R.W. and Visioli, F. (2006b), Minor Components of Olive Oil: Evidence to Date of Health Benefits in Humans. **Nutrition Review**, 64, S20-S30.

Cracowskia, A. and Ormezzanob, P. (2004), Isoprostanes, Emerging Biomarkers and Potential Mediators in Cardiovascular Diseases. **European Heart Journal**, 25, 1675-1678.

Daneman, D. (2006), Type 1 Diabetes. **Lancet**, 367, 847-858.

Deshpande, A., Harris-Hayes, M. and Schootman, M. (2008), Epidemiology of Diabetes and Diabetes Related Complications. **Physical Therapy**, 88(11), 1254-1264.

Department of Statistics, the Hashimite Kingdom of Jordan. (2009), Food Balance Sheet. DoS/HKJ, Amman.

Department of Statistics, the Hashimite Kingdom of Jordan. (2007), Food Balance Sheet. DoS/HKJ, Amman.

Department of Statistics, the Hashimite Kingdom of Jordan. (2007), Agricultural Census . DoS/HKJ, Amman.

Dunlop, M. (2000), Aldos Reductase and the Role of the Polyol Pathway in Diabetic Nephropathy. **Kidney International Suppl,** 77, S3-S12.

Eidi, A. and Eidi, M. (2009), Antidiabetic Effects of Sage (*Salvia Officinatis L.)* Leaves in Normal and Streptozotocin-Induced Diabetic Rats. **Diabetes and Metabolic Syndrome: Clinical Research and Reviews,** 3, 40-44.

Estruch, R., Martinez-Gonzalez .M.A., Corella, D., Salas-Salvado,J., Ruiz-Gutierrez, V., Covas, M.I, (2006), Effect of a Mediterranean-Style Diet on Cardiovascular Risk Factors: a randomized trial. **Annual Internal Medicin** , 145, 1-11.

Evans, J.L., Goldfine, I.D., Maddux, B.A. and Grodsky, G.M.(2003), Are Oxidative Stress- Activated Signaling Pathways Mediators of Insulin Resistance and beta-cell dysfunction? **Diabetes**, 52, 1-8.

FAOSTAT database, (2000).**http://apps.fao.org**.

Fito, M., Cladellas, M., D La Torre.R., Marti, J., Alcantara, M., Pujadas-Bastardes, M., Marrugate, J., Bruguera, J., Lopez-Sabater, M.C., Vilaa,J ., Covas, M.I(2005), Antioxidant Effect of Virgin Olive Oil in Patients with Stable Coronary Heart Disease: A Randomized, Crossover, Controlled, Clinical Trial. **Atherosclerosis**, 181, 149-158.

Fitó, M., De La Torre, R. and Covas, M. (2007), Olive Oil and Oxidative Stress. **Molecular .Nutrition &.Food Research ,** 51, 1215-1224.

Fossati, P. and Prencip, L.(1982), Serum Triglycerides Determined Colorimetrically with an Enzyme That Produces Hydrogen Peroxide. **Clinical Chemistry**, 28, 2077-2080.

Fuentes, F., Lopez-Miranda, J., Perez-Martinez, P., Jimenez, Y.,Marin, C., Gomez, P.,Fernandez, J.M., Caballero, J., Delqado-Lista, J. and Perez-Jimenez, F. (2008), Chronic Effect of a High-Fat Diet Enriched with Virgin Olive Oil and a Low-Fat Diet Enriched with alpha-Linolenic Acid on postprandial Endothelial Function in healthy men. **British Journal of Nutrition**, 100, 159-165.

Geiss, L.S., Herman, W.H., Smith, P.J.(1995), Mortality in Non-Insulin-Dependent Diabetes. In: Harris, M.I., Cowie, C.C., Stern, M.P, et al., Diabetes in America. 2nd ed.Bethesda, MD: National Institutes of health, National Institute of Diabetes and Digestive and kidney Diseases, (pp.233-257). NIH Publication 95-1468.

Gerstein, H.C. (2007).Point: If It Is Important to Prevent Type2 Diabetes, It Is Important to Consider All Proven Therapies within a Comprehensive Approach. **Diabetes Care**, 30, 432-434.

Gimeno, E., Castellote, A.I., Lamula-Raventose, R.M., De la Torre, M.C., Lopez-Sabater, M.C. (2002), The Effect of Harvest and Extraction Methods on the Antioxidant Content (Phenolics, α-tocopherol, and β-carotene) in Virgin Olive Oil. **Food Chemistry**, 78, 207-211.

Giron, M.D., Sanchez, F., Hortelano, P., Periago, J.L. and Suarez, M.D. (1999), Effects of Dietary Fatty Acids on lipid Metabolism in streptozotocin-Induced Diabetic Rats, **Metabolism**, 48, 455-460.

Glew, R.H. (2006), Lipid Metabolism II: Pathways of Metabolism of Special Lipids. In: Devlin, T.M., Biochemistry with Clinical Correlation, 6thed. (Pp.695-741) Canada: Wiley-liss., Hoboken,NJ.

Gupta, D., Raju., Prakash, R.J. and Baquer, N.Z.(1999).Change in the Lipid Profile, Lipogenic and Related Enzymes in the Liver of Experimental Diabetic Rats: Effect of Insulin and Vanadate. **Diabetes Research and Clinical Practice**, 46, 1-7.

Guthrie, R. and Guthrie, D. (2004), Pathophsiology of Diabetes Mellitus. **Critical Care Nursing Quarterly**, 27(2), 113-125.

Haddad, D.H. (2005), **Effect of Varying the Proportion of Olive Oil and Corn Oil in the Diet on Body Weight, Body Fat Content and Energy Balance in Rats.** Unpublished master thesis, University of Jordan, Amman, Jordan.

Hamden, K., Allouche, N., Damak, M., Elfeki,A.(2009), Hypoglycemic and Antioxidant Effects of Phenolic Extracts and Purified Hydroxytyrosol from Olive Mill Waste in Vitro and in rats, **Chemico-Biologyical Interactions**, 180,421-432.

Harris,R.A. and Crabb, D.W. (2006), Metabolic Interrelationships. In: Devlin, T.M., **Biochemistry with Clinical Correlations**, 6th ed. (pp.849-890) Canada: Wiley-liss., Hoboken, N.J.

Harris, R., Leininger, L.(1993), Preventive Care in Rural Primary Care Practice. Cancer, 72(3), 1113-1118.

Hu, F.B. (2004), The Mediterranean Diet and Mortality-Olive oil and beyond. **New England Journal of Medicine**, 340, 2595-2596.

Ibrahim, M.O. (2007), **Effect of Birhi Variety of Date Palm Fruits, *Phoenix Dactylitera* L. At the Tamr Stage on Serum Glucose Levels and Body Weight in Streptozotocin-Induced Diabetic Rats.** Unpublished master thesis, University of Jordan, Amman, Jordan.

Inoguchi , T., Li, P., Umeda ,F., Yu ,H.Y., Kakimoto, M., Imamura, M., Aoki, T., Etoh, T., Hashimoto, T., Naruse, M., Sano, H., Utsumi, H. and Nawata ,H.(2000), High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C--dependent activation of NAD(P)H oxidase in cultured vascular cells. **Diabetes**, 49, 1939-1945.

Itani, S.I., Ruderman, N.B., Schmieder, F. and Boden, G. (2002), Lipid-Induced Insulin Resistance in Human Muscle is Associated with Changes in Diacylglycerol, Protein Kinase C, and IκB-α. **Diabetes**, 51, 2005-2011.

Jemai, H., El Feki, A. and Sayadi, S. (2009), Antidiabetic and Antioxidant Effects of Hydroxytyrosol and Oleuropein from Olive Leaves in Alloxan-Diabetic Rats. **Agriculture Food Chemistry**, 57(14), 8798-8804.

Junod, A., Lambert, A. and Stauffacher, W. (1969), Diabetogenic Action of Streptozotocin Relationship of Dose to Metabolic Response. **Clinical Investigation**, 48, 2129-2139.

Kahan, B.B. and Flier, J.S. (2000), Obesity and Insulin Resistance. **Clinical Investigation.** 106, 473.

Kanarek, R. and Ho, L.(1984), Patterns of Nutrient Selection in Rats with Streptozotocin-Induced Diabetes. **Physiology and Behavior**, 32, 639-645.

Kaviarasan, S., Muniandy, S.,Qvist, R. and Ismail, I.S. (2009),F2 Isoprostane as Novel Biomarkers for Type 2 Diabetes: a Review. **Clinical Biochemistry and Nutrition**, 45, 1-8.

King,K.D., Jones, J.D., Warthen, J.(2005), Microvascular and Macrovascular Complications of Diabetes Mellitus, **American Journal Pharm Eduction**, 69,87.

Krzystek-Korpacka, M., Salmonowicz, B., Boehm, D., Berdowska, I., Zielinski, B., Patryn, E., Noczynska, A. and Gamian, A. (2008), Diagnostic Potential of Oxidative Stress Markers in Children and Adolescents with Type 1 Diabetes. **Clinical Biochemistry**, 41, 48-55.

LÁbbe, M.R., Trick, K.D. and Beare-Rogers, J.L. (1991), Dietary (n-3) Fatty Acids Effect on Rat Heart, Liver and Aorta Protective Enzyme Activities and Lipid Peroxidation. **Nutrition ,**121, 1131-1140.

Lenzen, S. (2008), The Mechanisms of Alloxan and Streptozotocin –Induced Diabetes. **Diabetologia**, 51, 216-226.

Lewis, G., Carpentier, A., Adeli, K. and Giacca, A. (2002), Disordered Fat Storage and Mobilization in the Pathogenesis of Insulin Resistance and type2 Diabetes. **Endocrine Reviews,** 23(2), 201-229.

Lopez, S., Bermudez, B., Pacheco, Y.M., Villar, J., Abia, R., Muriana, F.J.(2008), Distinctive Postprandial Modulation of beta Cell Function and Insulin Sensitivity by Dietary Fats: Monounsaturated Compared with Saturated Fatty Acids. **The American Journal of Clinical Nutrition**, 88(3), 638-644.

López-Miranda, J., Pérez-Jiménez, F., Ros, E., De Caterina, R., Badimon, L., Covas, M.I., Escrich, E., Ordovas, J.M., Soriguer, F., et al. (2010), Olive Oil and Health: Summary of the II International Conference on Olive Oil and Health Consensus Report, Jaén and Córdoba(Spain) 2008. **Nutrition, Metabolism& Cardiovascular Disease,** 20, 284-294.

Manghrani, M., Lemhadri, A., Zeggwagh, N., El Amraoui, M., Haloui, M., Jouad, H. and Eddouks, M. (2004), Effects of an Aqueous Extract of Triticum Repens on Lipid Metabolism in Normal and Recent-Onset Diabetic Rats. **Journal of Ethnopharmacology**, 90, 331-337.

Maritim, A. c., Sanders, R.A. and Watkins, J.B.(2003), Diabetes, Oxidative Stress, and Antioxidants: A Review. **Journal of Biochem Molecular Toxicology**, 17, 24-28.

Marin-Gallan, P., Carrascosa, A ., Gussinye, M.M. and Dominguez, C. (2003), Biomarkers of Diabetes-Associated Oxidative Stress and Antioxidant Status in Young Diabetic patients with or without Subclinical Complications. **Free Radical Biology Medicine ,** 34, 1563-1574.

Montonen, J., Knekt, P., Javinen, R. and Reunanen, A. (2004), Dietary Antioxidant Intake and Risk of Type 2 diabetes. **Diabetes Care**, 27, 362-366.

Montuschi, P., Peter, J., Roberts, J. (2004), Isoprostanes: Markers and Mediators of Oxidative Stress. **Journal of the Federation of Amierican Societies for Experimental Biology**, 18, 1791-1800.

Nakbi, A., Tayeb, W., Dabbou, S., Issaoui, M., Grissa, A.K., Attia, N. and Hammami, M. (2010), Dietary Olive Oil Effect on Antioxidant Status and fatty Acid Profile in the Erythrocyte of 2, 4-D-Exposed rats, **Lipids in Health and Disease,**9: 89.

Namkoong, C., Kim, M.S., Jang, P.G., Han, S.M., Park, H.S. and Koh, E.H. (2005), Enhanced Hypothalamic AMP-Activated Protein Kinase Activity Contributes to Hyerphagia in Diabetic Rats. **Diabetes**, 54, 63-68.

Naz, S., Siddiqi, R., Sheikh, H. and Sayeed, S.A.(2005), Deterioration of Olive, Corn, and Soybean Oils Due to Air, Light, heat and deep-frying. **Food Research International,** 38, 127-134.

Nazirog, I.M. and Butterworth, P.(2005), Protective Effects of Moderate Exercise with Dietary vitamin C and E on Blood Antioxidative Defens Mechanism in Rats with Streptozotocin-Induced Diabetes. **Can J Appl Physiol**, 30 (2), 172-185.

Nobar, M., Rahimi-Pour, A., Rahbani-Nobar, M., Adi-Beig, F. and Mirhashim, S. (1999), Total Antioxidant Capacity, Superoxide Dismutase and Glutathion Peroxidase in Diabetic Patients. **Medical Journal of Islamic Academy of Sciences**, 12(4), 109-114.

Ogasawara, A., Nakajima, A., Nakajima, F., Goto, K. and Yamazaki, M. (2008), Molecular Basis for Affected Cartilage Formation and Bone Union in Fracture Healing of the Streptozotocin-Induced Diabetic Rat. **Bone**, 43, 832-839.

Oliveras-López, M-J., Berná, G., Carneiro, E.M., Serrana, H.L-G., Martin, F., López, M.G.(2008), An Extra-virgin Olive Oil Rich in Polyphenolic Compounds has Antioxidant Effects in Of1 Mice. **Journal of Nutrition**, 138(6),1074-1078.

Owen, R.W., Mier, W., Giacosa, A., Hull, W.E., Spiegelhalder, B. and Bartsch, H.(2000), Phenolic Compounds and Squalene in Olive Oils: The Concentration and Antioxidant Potential of Total Phenols, Simple Phenols, Secoiridoids, Lignansand Squalene. **Food Chem Toxicol**, 38, 647-659.

Panagiotakos, D.B., Tzima, N., Pitsavos, C., Chrysohoou, C., Zampelas, A., Toussoulis, D. and Stefanadis, C.(2007), The Association Between Adherence to the Mediterranean Diet and Fasting Indices of Glucose Homoeostasis: the ATTICA Study. **Journal of American College Nutrition,** 26(1), 32-38.

Paniagua,J.A., Gallego de la Sacristana,A., Romero, I., Vidal-Puig, A., Latre, J.M., Sanchez, E., Perez-Martinez, P., Lopez-Miranda, J. and Perez-Jimenez, F..(2007a), Monounsaturated Fat-Rich Diet Prevents Cerntral Body Fat Distribution and Decreases Postprandial Adiponectin Expression Induced by a Carbohydrate-Rich Diet in Insulin Resistant Subjects**. Diabetes Care**, 30, 1717-1723.

Paniagua,J.A., Gallego de la Sacristana, A., Sanchez, E., Romero, I., Vidal-Puig, A., Berral, F.J., Escribano, A.,Moyano, M.J., Perez-Martinez, P., Lopez-Miranda, J. and Perez-Jimenez, F.(2007b), A MUFA-Rich Diet Improves Postprandial Glucose, Lipid and GLP-1 Responses in Insulin-Resistant Subjects. **Journal of the American College of Nutrition,**26, 434-444.

Paolisso, G., Gambardella, A., Tagliamonte, M.R., Saccomanno, F., Salvatore, T., Gualdiero, P., D'Onofrio, M.V. and Howard, B.V.(1996), Dose Free Fatty Acid Infusion Impair Insulin Action also Through an Increase in Oxidative Stress?. **Journal of Clinical Endocrinology and Metabolism,** 81(2), 4244-4248.

Pellegrrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S.,Bianchi, M. and Brighenti, F.(2003), The Antioxidant Capacity of plant Foods, Beverages and Oils Consumed in Italy Assessed by three Different in Vitro Assays, **The Journal of Nutrition**, 133, 2812-2819.

Pellegrini,N., Visioli, F., Buratti, S. and Brighenti, F. (2001), Direct Analysis of Total Antioxidant Activity of olive oil and Studies on the Influence of Heating. **Agriculture Food Chemistry**, 49, 2532-2538.

Perez-Jimenez, F., Ruano, J., Perez-Martinez, P., Lopez-Segura, F., Lopez-Miranda, J.(2007), The Influence of Olive Oil on Human Health: not a question of fat alone. **Molecular Nutrition & Food Research**, 51, 1199-1209.

Perona, J.S., Avella, M.(2006), Uptake of Triacylglycerol-rich Lipoproteins of Differing Triacylglycerol Molecular Species and Unsaponifiable Content by Liver Cells.B

Piwowar, A., Kordecka, M. and Warawas, M. (2007), AOPP and its Relations with Selected Markers of Oxidative/ Antioxidative System in Type2 Diabetes Mellitus. **Diabetes Research and Clinical Practice,** 77, 188-192.

Powers, S.K. and Jackson, M.J. (2008), Exercise-Induced Oxidative Stress: Cellular Mechanisms and Impact on Muscle Force Production. **The American Physiological Society**, 88, 1243-1276.

Puerta,C., Carrascosa-Samoral, M.P., Gacia-Luna, P.P., Lardone, P., Herrera, J.L., Fernandez-Montesinos, R.,Guerrero, J.M., Pozo, D.(2007). Melatonin is a Phytochemical in Olive oil. **Food Chemistry Journal,** 104, 609-612.

Psaltopoulou, T., Panagiotakos, D.B., Pitsavos, C., Chrysochoou, C., Detopoulou, P., Skoumas, J., Stefanadis, C.(2009). Dietary antioxidant Capacity is Inversely Associated with Diabetes Biomarkers: The ATTICA Study. **Nutrition, Metablism & Caediovascular Diseases,** xx,1-7.

Qasem, M.A.(2004), **Effect of Black cumin Seeds (*Nigella Sativa* L.) on Blood Glucose Levels and Body Weight in Streptozotocin-Induced Diabetic Rats,** Unpublished master thesis, University of Jordan, Amman, Jordan.

Quiles, J.L., Ochoa, J.J., Ramirez-Tortosa, C., Battino, M., Huertas, J.R., Martín, Y. and Mataix, J. (2004), Dietary Fat Type (Virgin Olive Oil vs. Sunflower Oils) Affects Age-Related Changes in DNA double-Strand-Breaks, Antioxidant Capacity and Blood Lipids in Rats, **Experimental Gerontology**, 39,1189-1198.

Rabinowitz, J.L. and Craig, R.G. (1989), Changes in Whole Body Lipid Composition in a Murine Model of Insulin-Dependent Diabetes Mellitus. **Metabolism**, 38, 777-780.

Reaven, P., Parthasarathy, S., Grasse, B.J., Miller, E., Steinberg, D. and Witztum, J.L. (1993), Effects of Oleate Rich and Linoleate Rich Diets on the Susceptibility of Low Density Lipoprotein to Oxidative Modification in Mildy Hypercholesterolemic Subjects, **Clinical. Invest,** 91, 668-676.

Reeves, P.G. (1997), Components of the AIN-93 Diets as Improvements in the AIN-76 Diet. **Journal of Nutrition**, 127, 838S-841S.

Riccardi, G., Giacco, R., Rivellese, A.A.(2004), Dietary Fat, Insulin Sensitivity and the Metabolic Syndrome. **Clinical Nutrition**, 23(4), 447-456.

Romaguera,D., Norat, T., Mouw, T., May, A.M., Bamia, C.,Slimani, N.,Travier, N., Besson, H., Luan, J., Wareham, N.,Rinaldi, S., et al. (2009), Adherence to the Mediterranean Diet is Associated with Lower Abdominal Adiposity in European Men and women. **Journal of Nutrition**, 139, 1728-1737.

Ros, E. (2003), Dietary Cis- Monounsaturated Fatty Acids and Metabolic Controlin Type 2 Diabetes. **The American Journal of Clinical Nutrition**, 78, 617S-625S.

Ruano, J.,Lopez-Miranda,J., Funtes, F., Moreno, J.A.,Bellido, C.,Perez-Martinez, P., et al.(2005), Phenolic content of Virgin Olive Oil Improves Ischemic Reactive Hyperemia in Hypercholesterolemic Patients. J Am Coll Cardiol, 46, 1864-1868.

Sakano, N., Wang, D., Takahashi, N., Wang, B., Sauriasari, R., Kanbara, S., Sato, Y., Takigawa, T., Takakil, J. and Ogino, N. (2009), Oxidative Stress Biomarkers and Lifestyles in Japanese Healthy People. **Clinical Biochemistry and Nutrition**, 44, 185-195.

Sauer, H. and Wartenberg, M. (2008), Circulation Isoprostanes: Gate Keepers in the Route from Oxidative Stress to Vascular Dysfunction. **American Health Association**, 103, 907-909.

Scott, J.A. and King, G. (2004), Oxidative Stress and Antioxidant Treatment in Diabetes. **Ann.N.Y.Acad.Sci**, 1031, 204-213.

Servili, M., Selvaggini, R., Esposto, S., Tatichcci, A., Montedoro, G.F.and Morozzi, G. (2004), Health and Sensory Properties of Virgin Olive Oil Hydrophilich Phenols: Agronomic and Technology Aspects of Production that Affect their Occurrence in the Oil. **Journal of Chromatography A**, 1054, 113-127.

Seghrouchni, I., Drai, J., Bannier, E., Riviere, J., Calmard, P., Garcia, I., Orgiazzi, J. and Revol, A. (2002), Oxidative Stress Parameters in Type I, Type II and Insulin-Treated Type2 Diabetes Mellitus; Insulin Treatment Efficiency. **Clinical Chimica Acta,** 321, 89-96.

Sicree, R. and Shaw, J.(2007), Type 2 Diabetes: An Epidemic or not, and why it is Happening? **Diabetes and Metabolic Syndrome: Clinical Research and Reviews**, 1, 75-81.

Sidel, J., Hagele, E.O., Ziegenhorn, J. and Wahlefeld, A.W.(1983), Reagent for the Enzymatic Determination of Serum Total Cholesterol with Improved Lipolytic Efficiency. **Clinical Chemistry**, 29, 1075-1080

Sirtori, G.R., Gatti, E.,Tremoli, E., Galli, G., Gain-Franceshi,G., Franees Chini, G. and Stragliotto, E.(1992), Olive Oil, Corn Oil, and n-3 Fatty acids Differently, Affect Lipid Lipoprotein, Platelets and Superoxide Formation in Type II Hypercholesterolemia. **American Journal of Clinical Nutrition**, 56(1), 113-122.

Stark, A.H. and Madar, Z. (2002), Olive Oil as a Functional Food: Epidemiology and Nutritional Approaches. **Nutrition Reviews**, 60, 170-176.

Sugiuchi,H., Uji, Y., Okabe, H., Irie, T., Uekama, K., Kayahara, N. and Miyauchi, K.(1995), Direct Measurement of High-Density Lipoprotein Cholesterol in Serum with Polyethylene Glyco-Modified Enzymes and Sulfated α-Cyclodextrin Sulfate. **Clinical Chemistry,** 41,717-723.

Sugiuchi, H., Irie, T.,Uji, Y., Ueno, T., Chaen, T., Uekama, K. and Okabe, H. (1998), Homogenous Assay for Measuring Low-Density Lipoprotein Cholesterol in Serum with Triblock Copolymer and α-Cyclodextrin Sulfate. **Clinical Chemistry,** 44,522-531.

Szkudelski, T. (2001), The Mechanism of Alloxan and Streptozotocin Action in β cells of the Rat Pancreases. **Pysiological Research,** 51, 255-259.

Szkudelski, T. and Szkudelska, K. (2002), Streptozotocin Induces Lipolysis in Rat Adipocytes in Vitro. **Physiological Research**, 51, 255-259.

Takasaki,Y.(2005), Serum Lipid levels and Factors Affecting Atherogenic Index in Japanese Children, **Physiological Anthropology and Applied Human Science**, 24(4), 511-515.

Trinder, P. (1969). Determination of Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. **Annuals of Clinical Biochemistry**, 6, 24-27.

Tur, M.J. (2004), The Quality of Fat: Olive Oil. **Arch Latinoam Nutrition**, 54(2), 59-64.

Ugochukwu, N.H., Bagayoko, N.D. and Antwi, M.E. (2004), The Effects of Dietary Caloric Restriction on Antioxidant Status and Lipid Peroxidation in Mild and Sever Streptozotocin-Induced Diabetic Rats. **Clinical Chimica Acta,** 348, 121-129.

Valls,R-M., Soler, A., Girona,J., Heras, M., Romero, M-P., Covas, M-I., Solá, R., Masana, L. and Motilva, M-J.(2010), Effect of the Long-Term Regular Intake of Virgin Olive Oil on the Phenolic Metabolites in Human Fasting Plasma, Journal of Pharmaceutical and Biomedical Analysis, 53.68-74.

Velasco, J. and Dobarganes, C. (2002), Oxidative Stability of Virgin Olive oil. **European Journal of Lipid Science and Technology**, 104, 661-676.

Vincent-Baudry, S., Defoort, C., Gerber, M., Bernard, M.C., Verger, P., Helal, O., Portugal, H., Planells, R., Grolier, P., Amiot-Carlin, M-J. and Lairon .P.V.D. (2005), The Medi-RIVAGE study: Reduction of Cardiovascular Disease Risk Factors After a 3-mo Intervention with a Mediterranean- Type Diet or Low- Fat Diet. **The American Journal of Clinical Nutrition**, 82, 964-971.

Virally, M., Blickle, J. and Girard, J. (2007), Type 2 Diabetes Mellitus: Epidemiology, Pathophysiology, Unmet Needs and Therapeutically Perspectives. **Diabetes and Metabolism,** 33,(4),231-244.

Visioli, F. and Galli, C. (2002), Biological Properties of Olive Oil Phytochemicals. **Critical Reviews in Food Science and Nutrition**, 42(3), 209-221.

Visioli, F., Grande, S., Bogani, P., Galli, C., in:Quiles, J.L., Ramirez-Tortosa, M.C. and Yaqoob, P(Eds), Olive Oil and Health, CAB international, Oxfordshire, uk, 2006.pp. 109-116.

Waterman, E. and Lockwood, B.(2007), Active components and Clinical Applications of Olive Oil , **Alternative Medicine Review**, 12(4), 331-342.

Wild, S., Roglic, G., Green, A., Sicree, R. and Kinge, H. (2004), Global Prevalence of Diabetes. **Diabetes Care**, 27(5), 1047-1053.

Will, J.C., Ford, E.S. and Bowman, B.A.(1999), Serum Vitamin C Concentrations and Diabetes: Findings From the Third National Health and Nutrition Examination Survey, 1988-1994.**American Journal of Clinical Nutrition,**70, 49-52.

Woeff, S.P. and Dean, R.T. (1987), Glucose Auto-Oxidation and Protein Modification. The Potential Role of Autooxidative Glycosylation in Diabetes. **Biochem**, 247, 243-250.

World Health Organization (WHO) (2006a), **Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia**. Report of the WHO consultation, Geneva.

World Health Organization (WHO) (2006b). **Guidelines for the Prevention, Management and Care of Diabetes Mellitus (Regional Office for the Eastern Mediterranean**. EMRO Technical Publications Series 32, Report of WHO Regional Office For the Eastern Mediterranean, Cairo, Egypt.

World Health Organization (WHO) (2009), **Media Centre Diabetes Fact Sheet N 312**. Geneva.

Yamada, R., Griggio, M.A. and Luz, J. (2002). Energy Balance of Pregnant Diabetic Rats. **British Journal of Nutrition**, 87, 509-515.

Yang, R., Guowei, L., Anlin, L., Zheng, J. and Shi, Y. (2006), effect of Antioxidant Capacity on Blood Lipid Metabolism and Lipoprotein Lipase Activity of Rats fed a High Fat Diet. **Nutrition,** 22, 1185-1191.

Yang, N., Zhao, M., Zhu, B., Yang, B., Chen, C., Cui, C. and Jiang, Y.(2008), Anti-diabetic Effects of Polysaccharides from Opuntia Monacantha Cladode in Normal and Streptozotocin-Induced Diabetic Rats. **Innovative Food Science and Emerging Technologies**, 9, 570-574.

Zhong, Y., Ahmed, S., Grupp, I.L. and Matlib, A.(2001), Altered SR Protein Expression Associated with contractile Dysfunction in Diabetic rat Hearts. **AJP. Heart and Circulatory Physiology**, 281, 1137-1147.

Zindah,M., Belbeisi, A., Walke, H. and Mokdad, A.H.(2008), Obesity and Diabetes in Jordan: Findings From the Behavioral Risk Factor Surveillance System, 2004. **Center for Diabetes Control and Prevention (CDC),** 5(1), 1-8.

Appendices

Table A1: Weekly body weight change of diabetic rats fed diets containing 4% and 8% of either virgin olive oil or stored oil preparations with peroxide values of 30 and 40 meqO_2/kg for five weeks [(1-3)].

Experimental groups		First week	Second week	Third week	Fourth week	Fifth week
Virgin olive oil (P.V= 10 meqO_2/kg)	4%	-21.1 ± 5.4[a]	7.9 ± 3[a]	-10.9 ± 6.7[a]	10.6 ± 4.7[a]	-5.9 ± 3.6 [ac]
	8%	-11.0± 4.6[a]	13.1 ± 3.7[a]	-6.7 ± 2.6[a]	7.6 ± 2.9[a]	-10.2 ±1.8[bc]
Stored olive oil, (P.V= 30 meqO_2/kg)	4%	-20.1 ± 8.2[a]	5.3 ± 3.5[b]	-12.4 ± 3.9[a]	14.4 ± 2.4[a]	6.8 ± 7.9[a]
	8%	-10.4 ± 8.4[a]	11.8 ± 2.4[a]	-7.5 ± 4.5[a]	4.9 ± 5.2[a]	-5.4 ± 5.1[ac]
Stored olive oil (P.V= 40 meqO_2/kg)	4%	-18.0 ± 10[a]	13.9 ± 5.5[a]	-12.2 ± 6.4[a]	4.2 ± 3.7[a]	-7.6 ± 4.1[bc]
	8%	-13.5 ± 2.5[a]	16.3 ± 2.9 [a]	-10.2 ± 3.2[a]	11.5 ± 5.1[a]	-9.6 ± 3.6[bc]

(1) Each value represented as mean ± SEM in grams.
(2) Values in a column with different superscripts are significantly different ($p<0.05$).
(3) P.V: Peroxide value.

Table A2: Weekly food intake of diabetic rats fed diets containing 4% and 8% of either virgin olive oil or stored oil preparations with peroxide values of 30 and 40 meqO_2/kg for five weeks [(1-3)].

Experimental groups		First week	Second week	Third week	Fourth week	Fifth week
Virgin olive oil (P.V= 10 meqO_2/kg)	4%	219 ± 5.6^{a}	197.4 ± 6.3^{a}	204.4 ± 6.8^{a}	192.4 ± 10.1^{a}	205.6 ± 6.9^{a}
	8%	199.8 ± 3.4^{ac}	197.4 ± 6.1^{a}	202 ± 7.9^{a}	176.4 ± 6.6^{a}	180.4 ± 7.2^{a}
Stored olive oil (P.V= 30 meqO_2/kg)	4%	201.5 ± 7.1^{ac}	204.3 ± 7.9^{a}	197.4 ± 7.7^{a}	198 ± 8.1^{a}	189.6 ± 9.5^{a}
	8%	193.7 ± 10.1^{bc}	200.8 ± 10.1^{a}	183.5 ± 8.9^{a}	182.4 ± 7.6^{a}	189.7 ± 8.1^{a}
Stored olive oil (P.V= 40 meqO_2/kg)	4%	197.4 ± 6.4^{bc}	202.7 ± 9.8^{a}	187.6 ± 12.1^{a}	171.9 ± 11.8^{a}	188.9 ± 14.5^{a}
	8%	193.1 ± 9.9^{bc}	192.3 ± 2.9^{a}	183.4 ± 8.8^{a}	181.2 ± 11.4^{a}	182.4 ± 10.9^{a}

(1) Each value represented as mean ± SEM, in grams.
(2) Values in a column with different superscripts are significantly different ($p<0.05$).
(3) P.V: Peroxide value.

Table A3: Weekly water intake of diabetic rats fed diets containing 4% and 8% of either virgin olive oil or stored oil preparations with peroxide values of 30 and 40 meqO_2/kg for five weeks [(1-3)].

Experimental groups		First week	Second week	Third week	Fourth week	Fifth week
Virgin olive oil (P.V= 10 meqO_2/kg)	4%	1.25 ± 0.08^{a}	1.22 ± 0.09^{a}	1.22 ± 0.08^{a}	1.24 ± 0.07^{a}	1.18 ± 0.06^{a}
	8%	1.18 ± 0.03^{a}	1.15 ± 0.06^{a}	1.1 ± 0.08^{ab}	0.99 ± 0.08^{bc}	0.89 ± 0.04^{b}
Stored olive oil (P.V= 30 meqO_2/kg)	4%	1.17 ± 0.12^{a}	1.16 ± 0.12^{a}	1.06 ± 0.1^{ab}	1.12 ± 0.07^{ac}	0.97 ± 0.07^{b}
	8%	1.06 ± 0.18^{a}	1.12 ± 0.14^{a}	1.07 ± 0.09^{ab}	0.97 ± 0.11^{bc}	0.96 ± 0.06^{b}
Stored olive oil (P.V= 40 meqO_2/kg)	4%	1.17 ± 0.09^{a}	1.12 ± 0.13^{a}	0.98 ± 0.1^{ab}	1.04 ± 0.09^{ac}	0.89 ± 0.07^{b}
	8%	1.1 ± 0.08^{a}	1.05 ± 0.05^{a}	0.9 ± 0.08^{b}	0.94 ± 0.08^{bc}	0.93 ± 0.08^{b}

(1) Each value represented as mean ± SEM, in liters.
(2) Values in a column with different superscripts are significantly different ($p<0.05$).
(3) P.V: Peroxide value.

Table A4: Weekly food efficiency ratio of diabetic rats fed diets containing 4% and 8% of either virgin olive oil or stored oil preparations with peroxide values of 30 and 40 meqO_2/kg for five weeks [(1-3)].

Experimental groups		First week	Second week	Third week	Fourth week	Fifth week
Virgin olive oil (P.V= 10 meqO_2/kg)	4%	-9.6 ± 2.6[a]	3.9 ± 1.5[a]	-8.7 ± 1.8[a]	5.2 ± 2.2[a]	-2.9 ± 1.7[ac]
	8%	-6.1 ± 2.9[a]	6.7 ± 1.8[a]	-3.2 ± 1.3[a]	4.4 ± 1.7[a]	-5.7 ±0.9[bc]
Stored olive oil (P.V= 30 meqO_2/kg)	4%	-9.7 ± 4.5[a]	2.9 ± 2.1[a]	-5.9 ± 2.1[a]	7.5 ± 1.2[a]	2.7 ± 3.7[a]
	8%	-5.6 ± 5.4[a]	6.3 ± 1.7[a]	-3.8 ± 2.8[a]	2.9 ± 2.8[a]	-2.8 ± 3.1[ac]
Stored olive oil (P.V= 40 meqO_2/kg)	4%	-7.8 ± 5.3[a]	6.8 ± 2.9[a]	-6.1 ± 3.4[a]	2.4 ± 2.3[a]	-3.8 ± 2.5[ac]
	8%	-6.9 ± 1.4[a]	8.5 ± 1.5[a]	-6.3 ± 2.2[a]	5.9 ± 2.6[a]	-4.7 ± 1.7[bc]

(1) Each value represented as mean ± SEM.
(2) Values in a column with different superscripts are significantly different ($p<0.05$).
(3) P.V: Peroxide value.

تأثير زيت الزيتون البكر على السّعة المضادة للأكسدة و وضبط غلوكوز و ليبدات الدم في جرذان بالغة مصابة بمرض السكري المحرّض بالستريبتوزوتوسين.

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ملخص

أجريت هذه الدراسة بهدف تقيم تأثير إضافة نسب متفاوتة من زيت الزيتون البكر و زيت الزيتون المخزن كمستحضر غذائي على محتوى مصل الدم من السعة المضادة للأكسدة و الغلوكوز و الشحميات و البروتينات الشحمية في جرذان مصابة بمرض السكري. تم أحداث السكري جزئيا في الجرذان عن طريق حقنها في غشاء تجويف البطن بمادة الستريبتوزوتوسين (32.25مغ \ كغم). تم تعريض زيت الزيتون البكر إلى ظروف التخزين المسرعة للحصول على زيت يحتوي على 1.5x و2.0x من أعلى قيمة للبيروكسايد المسموح بها لزيت الزيتون البكر حسب المواصفة الأردنية. و بالتالي كانت المجموعات المستخدمة في هذه التجربة, زيت الزيتون البكر و زيت الزيتون المخزن المحتوي على 30, 40 meqO_2\kg من قيمة البيروكسايد. تم تقسيم ثمانية وأربعين ذكرا من جرذان سبراغ- داولي (4.6 ± 217.2غم) إلى ست مجموعات كل مجموعة تحتوي على ثمانية جرذان. تم إعطأوهم غذاء يحتوي على إما4% أو 8% من زيت الزيتون البكر أو زيت الزيتون المخزن(1.5x P.V and 2.0x P.V) لمدة خمس أسابيع. أما بالنسبة لمحتوى مصل الدم من الغلوكوز و مجموعة الكوليستيرول و البروتينات الشحمية عالية الكثافة و البروتينات الشحمية منخفضة الكثافة و الغليسيريدات الثلاثية فتم قياسها عن طريق التحليل الأنزيمي-اللوني . وتم تسجيل وزن الجسم ووزن الكبد و وكمية الطعام المتراكمة و كمية الماء المستهلكة. كما تم قياس مقدار الحموضة , البيروكسايد و السعة المضادة للأكسدة لكل من زيت الزيتون البكر و زيت الزيتون المخزن المعد مسبقا.

وكانت نتائج البيروكسايد لزيت الزيتون البكر(10± 0.01 meqO_2\kg) ولزيت الزيتون المخزن 30 و40 meqO_2\kg لكل من 1.5x P.V و2.0x P.V على التوالي. وكانت نتائج الحموضة لزيت الزيتون البكر (0.98±0.02 Oleic acid%) التي ازدادت مع زيادة قيمة البيروكسايد (1.52±0.01 and 1.24±0.01 Oleic acid%) لكل من 1.5x P.V و 2.0x P.V على التوالي. أما بالنسبة لمجموع السعة المضادة للأكسدة فلوحظ انخفاض قيمة السعة المضادة للأكسدة مع ازدياد قيمة البيروكسايد, حيث كانت في زيت الزيتون البكر(2.22±0.02mmol\l) و كانت 2.15±0.02 و 2.05±0.01 لكل من 1.5x P.V و2.0x P.V على التوالي.

لم يلاحظ أي تأثير ذو قيمة معنوية($P>0.05$) لزيت الزيتون البكر والمخزن 4% و8% على كل من مقدار التغير في وزن الجسم و كمية الغذاء التراكمية المستهلكة و كمية الماء التراكمية المستهلكة و كفاءة الغذاء. بغض النظر عن نسبة الزيت أو قيمة البيروكسايد في الزيت, فكان لهم نفس النتيجة. وجد أن الوزن النسبي للكبد عند مجموعة الجرذان التي تناولت زيت الزيتون المخزن و الذي كانت فيه قيمة البيروكسايد تساوي30 meqO_2\kg بنسبة 4% (3.5±0.15) كانت أقل معنويا من مجموعة الجرذان التي تغذت على زيت الزيتون البكر بنسبة 4%(0.09 ± 4.1) ومن المجموعة التي تغذت على الزيت المخزن والذي كانت فيه قيمة البيروكسايد 30 meqO_2\kg

بنسبة 8% (4.1±0.03). من ناحية أخرى, لم يكن هناك أي تأثير ذو قيمة معنوية (P>0.05) لتغذية الجرذان على زيت الزيتون البكر ووزيت الزيتون المخزن المعد مسبقا بنسبة 4%و 8% على محتوى مصل الدم من الغلوكوز و مجموعة الكوليستيرول و البروتينات الشحمية عالية الكثافة و البروتينات الشحمية منخفضة الكثافة و الغليسيريدات الثلاثية و نسبة البروتينات الشحمية عالية الكثافة إلى البروتينات الشحمية منخفضة الكثافة و نسبة البروتينات الشحمية علية الكثافة إلى الغليسيريدات الثلاثية و مؤشر إحداث تصلب الشرايين. ونفس النتيجة تم الحصول عليها عند المقارنة بين مجموعات التجارب بغض النظر عن كل من نسبة الزيت و قيمة البيروكسايد . أما بالنسبة لمحتوى مصل الدم على السعة المضادة للأكسدة فكانت تزداد بشكل ملحوظ مع ازدياد قيمة البيروكسايد. ولوحظ أن مجموعة الجرذان التي تناولت الزيت المخزن الذي فيه قيمة البيروكسايد تساوي $40meqO_2\backslash kg$ بنسبة 8% كانت أعلى معنويا (P<0.05) (1.18±0.02mmol\l) من مجموعتي الجرذان التي تناولت زيت الزيتون البكر بنسبة 4% (1.05±0.03mmol) والزيت المخزن الذي فيه قيمة البيروكسايد $30\ meqO_2\backslash kg$ وبنسبة 4% (1.07±0.03mmol\l). أما مجموعة الجرذان التي تناولت 4% من زيت الزيتون البكر كان محتوى مصل الدم من السعة المضادة للأكسدة اقل من المجموعة التي تناولت زيت مخزن قيمة البيروكسايد في $30meqO_2\backslash kg$ بنسبة 8% . ووجد هناك علاقة ايجابية بين السعة المضادة للأكسدة وكل من مجموعة الكوليستيرول(r = 0.39, P<0.01) و الغليسيريدات الثلاثية(r =0.35, P<0.05) وكان هناك علاقة سلبية بين السعة المضادة للأكسدة و كمية الغذاء المستهلكة(r =-0.35, P<0.05) وكمية الماء المستهلكة.(r =-0.42, P<0.05) . وارتبط مستوى السكر بالمصل ارتباطا ايجابيا مع كل من كمية الغذاء المستهلكة (r =0.33, P<0.05) و مع كمية الماء المستهلكة (r =0.43, P<0.01) وارتبط سلبيا مع مقدار التغير في الوزن (r =-0.39, P<0.01) و مع كفاءة الغذاء (r =-0.42, P<0.01). أما بالنسبة لكمية الغذاء المستهلكة فكان هناك علاقة ايجابية مع كمية الماء المستهلكة(r=0.81,P<0.01) و ارتبطت مجموعة الكوليستيرول و البروتينات الشحمية منخفضة الكثافة ارتباطا سلبيا مع مقدار التغير في وزن الجسم (r =-0.36, P<0.05; r =-0.42, P<0.01)على التوالي , و مع كفاءة الغذاء (r =-0.34, P<0.05; r =-0.42, P<0.01) على التوالي.

نستنتج من هذه الدراسة أن زيت الزيتون البكر أو المخزن لم يكن له تأثير واضح على محتوى مصل الدم من الغلوكوز و مجموعة الكوليستيرول و البروتينات الشحمية عالية الكثافة و البروتينات الشحمية منخفضة الكثافة و الغليسيريدات الثلاثية على الجرذان مصابة بمرض السكري المحرض بالستريبتوزوتوسين غير مسيطر عليها. ونفس الشيء بالنسبة لوزن الجسم, وكمية الطعام المستهلكة و كمية الماء المستهلكة.زيادة قيمة البيروكسايد و الحموضة للزيت رافقها انخفاض في قيمة السعة المضادة للأكسدة لزيت وهذا حسن من محتوى مصل الدم على السعة المضادة للأكسدة في الجرذان المصابة بالسكري.